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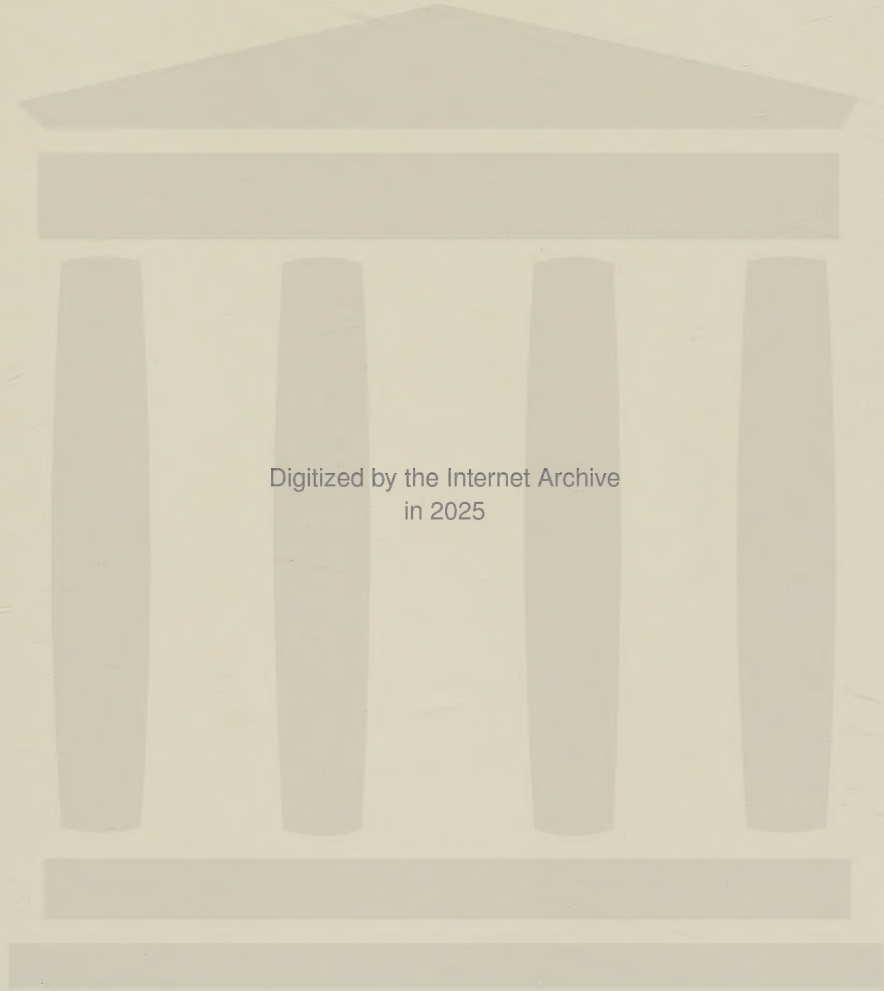

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Contribution No. 252

GENERALIZATION FOR CYLINDERS OF PRANDTL'S LINEAR ASSUMPTION FOR MIXING LENGTH

BY

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GENERALIZATION FOR CYLINDERS OF PRANDTL'S LINEAR ASSUMPTION FOR MIXING LENGTH*

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INTRODUCTION

In atmospheric and oceanic boundary-layer problems certain crucial quantities have defied direct measurement. One of these is the shearing stress between the air and the ground or between the air and the ocean surface. This has been computed indirectly: on the one hand from the frictional retardation of the wind as manifested in the transport of air in the direction of the horizontal pressure gradient (across the isobars); and on the other hand from the slope of the sea surface produced by the wind in constricted parts of the ocean and from the movement of sea ice. Another such quantity is evaporation from natural land and water surfaces, which has been estimated from the thermal energy used in vaporization as computed from the other terms in the heat balance for the ground or water. The values resulting from these various methods are not of high accuracy.

A method sometimes used in the calculation of these quantities utilizes the controlled experiments on turbulent flow which have been obtained in hydraulic and aerodynamic laboratories. The experiments which lend themselves most readily to this application are those on the flow of fluids through long circular cylinders. Due to the relative simplicity of the model, the velocity distribution and axial pressure gradient can be measured with great accuracy, and the latter gives the shearing stress at any distance from the axis. Except near the entrance the mean velocity at a point depends on one coordinate alone, the distance from the axis. In this important respect the flow is similar to a horizontally uniform wind, in which the mean velocity depends only on the distance from the ground, a boundary essentially in the form of a plane of infinite extent.

The infinite plane and the infinitely long cylinder of small radius are nevertheless boundaries of definitely different geometric pattern. In applying to one the results from the other it is therefore necessary to be

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sure that the theory involved can be applied satisfactorily to both models.

The approach followed here is the very fruitful one due primarily to Prandtl and von Kármán. By means of their general theory the quantitative observations of the complicated pattern of turbulent flow can be expressed largely in terms of a single constant, the universal turbulence constant. The atmospheric applications give the shearing stress and evaporation as proportional to the square of this constant. The value of the constant obtained experimentally depends, however, on the particular form of the general theory employed. Two previous forms of the general theory, one giving the constant as 0.40 and the other as 0.31, are examined below and found unsatisfactory. A third form, which is apparently in accord with the observations, is advanced here and yields 0.45 as the value of the universal turbulence constant.

GENERAL CONSIDERATION OF TURBULENT FLOW IN A CIRCULAR CYLINDER

For the steady mean flow of an incompressible homogeneous fluid in a long circular cylinder the mean velocity is everywhere parallel to the axis and is therefore expressed completely by the one component u. The mean velocity varies only with distance z from the wall, or distance r from the axis. In terms of the radius r_0 of the cylinder,

$$z + r = r_0.$$

The *relative distance from axis* may be written as

$$k = \frac{r}{r_0} = 1 - \frac{z}{r_0} \qquad (1).$$

The effective shearing stress τ across any concentric cylindrical surface varies linearly with distance from the axis, which follows from the balance between normal and shearing stresses on any concentric fluid cylinder. If τ_0 is the wall stress,

$$\tau = \tau_0 k \qquad (2).$$

The *friction velocity*, defined as

$$u_* = \sqrt{|\tau|/\rho}, \qquad u_{*0} = \sqrt{|\tau_0|/\rho} \qquad (3),$$

where ρ is density, therefore varies as

$$u_* = u_{*0}\sqrt{k} \qquad (4).$$

The effective shearing stress is composed of two terms,

$$\tau = \mu \frac{du}{dz} - \rho \overline{w'u'} \qquad (5),$$

where μ is dynamic viscosity and $\overline{w'u'}$ is the mean product of the instantaneous velocity deviations in the directions normal to the boundary and parallel to the axis. Except in the vicinity of the boundary the first term is negligible for well-developed turbulent flow.

In order to evaluate the second term, the Reynolds stress, Prandtl[7] has introduced the *mixing length* l, which may be defined by

$$-\rho\,\overline{w'u'} = \rho\, l^2 \left|\frac{du}{dz}\right| \frac{du}{dz} \tag{6}.$$

Except near the boundary, then,

$$u_* = l\,\frac{du}{dz} = -\frac{l}{r_0}\frac{du}{dk} \tag{7}.$$

Combining equations (4) and (7) gives

$$\frac{l}{r_0} = -\,u_{*0}\,\frac{\sqrt{k}}{du/dk} \tag{8},$$

by means of which the mixing length can be computed from the observed velocity distribution and wall stress. For sufficiently high Reynolds numbers Nikuradse[5,6] has found by this means that the *relative mixing length* l/r_0 depends only on the relative distance from axis. His empirical equation,

$$l/r_0 = 0.14 - 0.08\,k^2 - 0.06\,k^4 \tag{9},$$

identical for both smooth and rough pipes, is shown graphically in FIGURE 1.

Although equation (8) is not always applicable close to the wall, it may be integrated to give the distribution of *velocity defect* $u_m - u$, where u_m is the mean velocity at the axis, throughout the rest of the cylinder,

$$\frac{u_m - u}{u_{*0}} = \int_0^k \frac{\sqrt{k}}{l/r_0}\,dk \tag{10}.$$

It has been found experimentally that for well-developed turbulent flow this ratio $(u_m - u)/u_{*0}$ is apparently a universal function of the relative distance from axis, the function extending close to the wall (von Kármán, 1934, p. 6). Some experimental points showing this function are plotted in FIGURE 2.

By studying the distribution of velocity defect instead of the distribution of velocity itself, one avoids the complicated conditions close to the boundary, where also the observations are inherently less accurate. Furthermore, the universal character found empirically for the velocity defect indicates that the central region of a cylinder offers the simplest proving ground for theory.

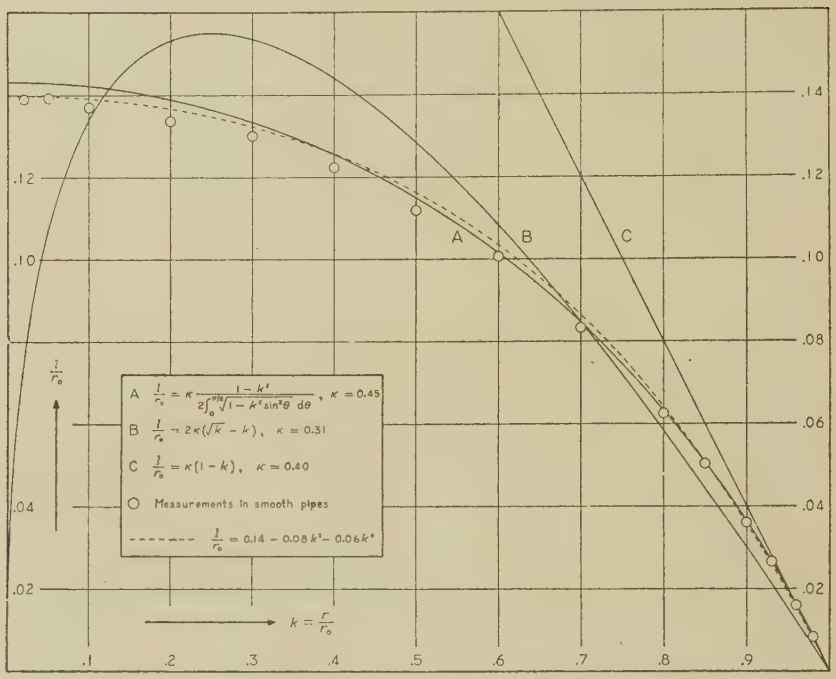

FIGURE 1. Relative mixing length as a function of the relative distance from axis of a circular cylinder.

A theory for the velocity distribution may be expressed in terms of the mixing length, and each of the three variations below introduces the universal turbulence constant. The satisfactory theory for the distribution of mixing length must not only be applicable for an infinite plane boundary but also agree with equation (9) for a circular cylinder. Or better, since the empirical equation (9) was determined from small differences of observed velocities, when inserted in equation (10) the mixing-length theory should give a function in close agreement with the directly observed distribution of the ratio $(u_m - u)/u_{*0}$.

LINEAR DISTRIBUTION OF MIXING LENGTH

Prandtl (1932, p. 189) found that in the immediate vicinity of a solid boundary the mixing length is, very nearly, simply a linear function of the shortest distance from the boundary. The proportionality factor is the *universal turbulence constant* κ. With an additive term in the form introduced by Rossby and Montgomery (1935, p. 5)

$$l = \kappa (z + z_0) \qquad (11).$$

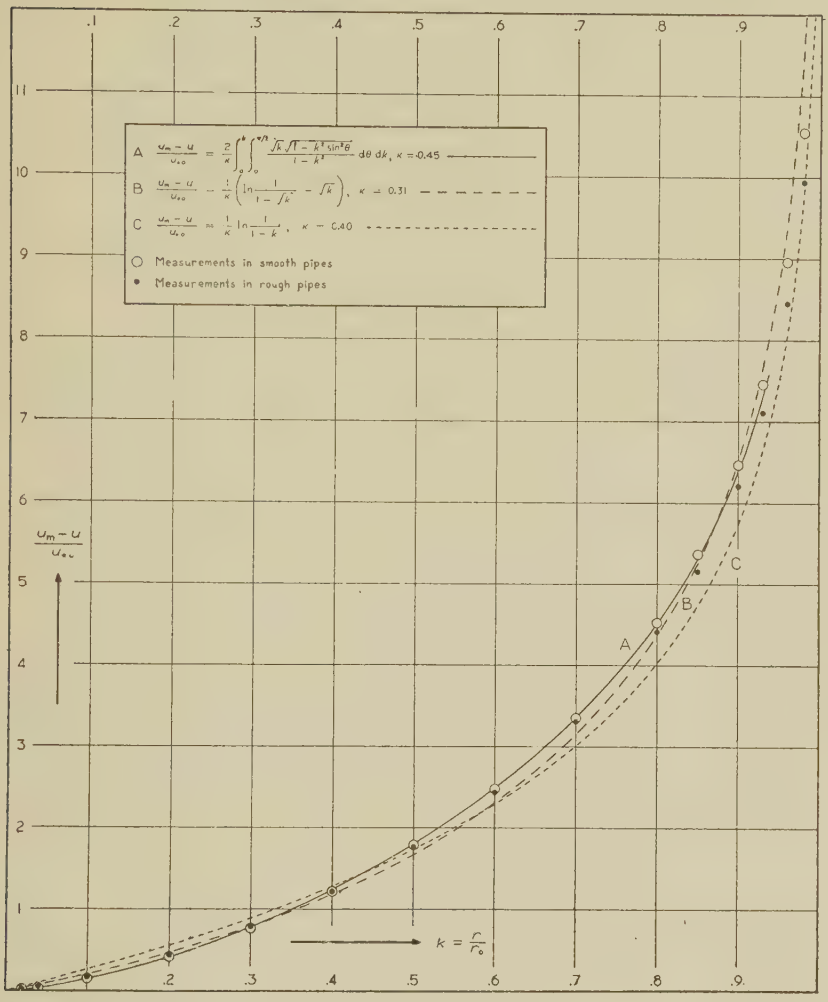
A $\frac{u_m - u}{u_{*0}} = \frac{2}{\kappa}\int_0^k \int_0^{\pi/2} \frac{\sqrt{k}\sqrt{1-k^2\sin^2\theta}}{1-k^2}\, d\theta\, dk$, $\kappa = 0.45$
B $\frac{u_m - u}{u_{*0}} = \frac{1}{\kappa}\left(\ln\frac{1}{1-\sqrt{k}} - \sqrt{k}\right)$, $\kappa = 0.31$
C $\frac{u_m - u}{u_{*0}} = \frac{1}{\kappa}\ln\frac{1}{1-k}$, $\kappa = 0.40$
Measurements in smooth pipes
Measurements in rough pipes
$\frac{u_m - u}{u_{*0}}$
$k = \frac{r}{r_0}$
A
B
C

FIGURE 2. Ratio of velocity defect to friction velocity as a function of the relative distance from axis of a circular cylinder.

The length z_0, which for a hydrodynamically rough boundary has been found to depend on the nature of the boundary alone, may be called the *roughness length.*

From equation (7) it follows that

$$\frac{du}{u_*} = \frac{1}{\kappa}\frac{dz}{z + z_0}.$$

For a boundary layer within which the variation of friction velocity is

negligible this gives on integration, assuming the mean velocity to vanish at $z = 0$,

$$\frac{u}{u_{*0}} = \frac{1}{\kappa} \ln \frac{z + z_0}{z_0} \qquad (12).$$

Except for the thin layer next to the boundary where z_0 is comparable with z, the last equation may be written

$$\frac{u}{u_{*0}} = \frac{1}{\kappa} \ln \frac{z}{z_0} \qquad (13),$$

given first by Prandtl.[8] This logarithmic distribution of wind above the ground agrees with observation within experimental error when the air is in neutral hydrostatic equilibrium (Prandtl, 1932; Lettau, 1939, p. 72).

The assumption of linear distribution of mixing length and the disregard of the variation of friction velocity are in definite disagreement with conditions in a circular cylinder except within a boundary layer extending perhaps a tenth of the distance to the axis. If one nevertheless applies the last equation all the way to the axis, it gives

$$\frac{u_m - u}{u_{*0}} = \frac{1}{\kappa} \ln \frac{1}{1 - k} \qquad (14).$$

Surprisingly enough, this agrees rather well with the observed distribution. The reason that the two false assumptions lead to such a good result is that the basic differential equation (7) contains the ratio of friction velocity to mixing length, both of which, by virtue of the assumptions, are increasingly too large on approaching the axis. The two errors therefore cancel to some extent. It may be noted that the resulting expression gives finite shear at the axis, which is physically impossible.

A value of 0.40 for the universal turbulence constant gives the best agreement between this theoretical expression and observation (Rouse, 1938, p. 244). Curves C in FIGURES 1 and 2 show how good the agreement is.

The generally accepted value for the universal turbulence constant is 0.40, derived from a somewhat different treatment of the problem—using actual velocity instead of velocity defect—but still involving constant friction velocity and linear increase of mixing length well into the central portion of the cylinder (von Kármán, 1934, p. 9). For application over a plane boundary this determination is seen to be unsatisfactory.

MIXING LENGTH IN TERMS OF FIRST AND SECOND DERIVATIVES OF MEAN VELOCITY

By stipulating similarity of the pattern of turbulent flow, von Kármán (1930, p. 63) concluded that the mixing length is given by

$$l = \kappa \left| \frac{du}{dz} \Big/ \frac{d^2u}{dz^2} \right| \qquad (15),$$

where κ is again the universal turbulence constant. From equation (7) it follows that

$$- u_* \frac{d^2u}{dz^2} \Big/ \left(\frac{du}{dz}\right)^2 = \kappa.$$

For a boundary layer within which the variation of friction velocity is negligible this gives on integration

$$u_{*0} \Big/ \frac{du}{dz} = \kappa\,(z + \text{const.}).$$

The left side is the mixing length, so equation (15) reduces to equation (11) and therefore this assumption for mixing length is applicable for an infinite plane boundary.

At the axis of a small circular cylinder the shear vanishes but the second derivative of velocity is finite. According to equation (15) the mixing length would therefore vanish, which is contrary to observation. It is thus seen at once that this assumption is not suitable for flow in circular cylinders.

Taking into account the variation of friction velocity and introducing the relative distance from axis, the differential equation for the velocity distribution becomes

$$- u_{*0} \frac{d^2u}{dk^2} \Big/ \left(\frac{du}{dk}\right)^2 = \frac{\kappa}{\sqrt{k}} \qquad (16)$$

At the wall the shear is relatively great, hence as a boundary condition for the first integration the shear is assumed infinite at the wall, giving

$$- \frac{1}{u_{*0}} \frac{du}{dk} = \frac{1}{2\kappa} \frac{1}{1 - \sqrt{k}} \qquad (17).$$

This gives finite shear at the axis, so it is valid neither at the axis nor at the wall. According to this relation the relative mixing length is

$$l/r_0 = 2\kappa\,(\sqrt{k} - k) \qquad (18).$$

It vanishes at the axis and at the wall, and is maximum at a quarter of the distance from axis to wall.

Integration of equation (17) gives, as the equation for the velocity defect,

$$\frac{u_m - u}{u_{*0}} = \frac{1}{\kappa}\left(\ln \frac{1}{1 - \sqrt{k}} - \sqrt{k}\right) \qquad (19).$$

In order to fit this to the observed distribution of velocity defect von Kármán (1934, Fig. 8) used 0.31 for the universal turbulence constant. With this value, equations (18) and (19) are drawn in FIGURES 1 and 2 respectively as curves B.

A NEW HYPOTHESIS FOR THE MIXING LENGTH

It is desirable to seek a more generally applicable assumption for the mixing length. For a plane boundary this assumption should reduce, as does von Kármán's, to a linear distribution.

Prandtl's geometrical assumption for mixing length can be generalized so that the dependence is not merely on the shortest distance to the boundary. It is reasonable to expect that at a given shortest distance to the boundary the mixing length is, for instance, less in a circular cylinder than near a plane surface, because the boundary is nearer in other than the shortest direction to it.

The models concerned are cylinders of infinite extent with the flow parallel to the axis, the infinite plane being merely a limiting form of circular cylinder. The shape of the boundary is therefore determined by the cross section of the cylinder. It may be assumed that at a point within the cylinder the mixing length depends on the distance to the wall in all directions on the cross-sectional plane. It would not be appropriate to use a function of simply the average distance, since this would be infinite near a plane boundary. One may make the hypothesis, however, that the mixing length is a linear function of the reciprocal of the average "nearness," in the plane normal to the cylinder, to the cylindrical wall.

To state this more precisely, consider the cross section of any cylinder as illustrated in FIGURE 3,a. In this plane let s be the distance from a point to the wall in the direction θ, this angle being measured from any chosen direction. Then the hypothesis is that the mixing length at this point is

$$l = \kappa\left[\frac{2}{\int_0^{2\pi} \frac{d\theta}{s}} + z_0\right] \qquad (20),$$

where κ and z_0 have the same significance as before.

Suppose the wall is a plane at distance z from the point in question, as

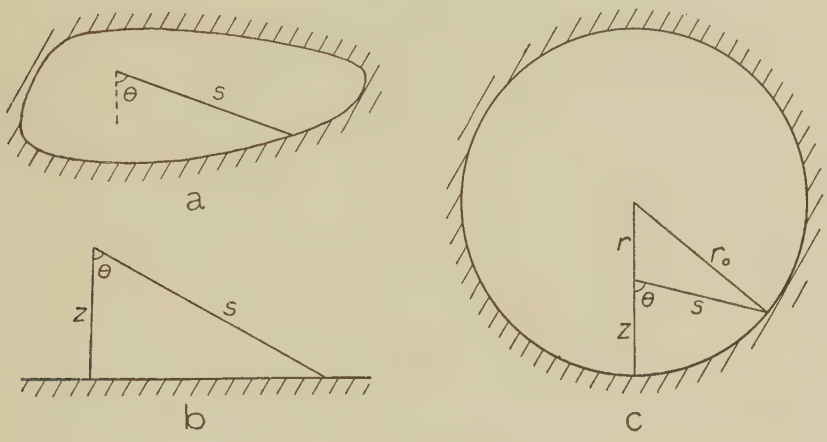

FIGURE 3. Cross sections of cylinders.

shown in FIGURE 3,b. Then the direction may be measured from the perpendicular to the wall, and

$$\int_0^{2\pi} \frac{d\theta}{s} = \frac{1}{z}\int_{-\pi/2}^{\pi/2} \cos\theta \, d\theta = \frac{2}{z}.$$

Thus equation (20) reduces to equation (11), so the assumption is applicable for a plane boundary.

Between two flat plates distant by $2\ r_0'$ the mixing length would be

$$l = \kappa\,[z(1 - z/2r_0') + z_0] \qquad (21).$$

At the mid-plane this gives the relative mixing length as

$$\frac{l_m}{r_0'} = \frac{\kappa}{2} \qquad (22),$$

neglecting the roughness length.

The relative mixing length at the axis of a circular cylinder is found immediately to be

$$\frac{l_m}{r_0} = \frac{\kappa}{\pi} \qquad (23).$$

Hence, from the empirical expression for relative mixing length, equation (9), the universal turbulence constant would be

$$\kappa = 0.14\,\pi = 0.44.$$

The empirical determination of relative mixing length near the axis is rather uncertain, however, and the velocity defect will be found better fitted by the value 0.45.

For an arbitrary point within a circular cylinder it is seen by reference to the triangle in FIGURE 3,c that

$$s = \sqrt{r_0^2 - r^2 \sin^2(\pi - \theta)} + r \cos(\pi - \theta).$$

Hence

$$s/r_0 = \sqrt{1 - k^2 \sin^2\theta} - k \cos\theta,$$

and

$$\frac{r_0}{s} = \frac{\sqrt{1 - k^2 \sin^2\theta} + k \cos\theta}{1 - k^2}.$$

Now

$$\int_0^{2\pi} \cos\theta \, d\theta = 0$$

and the elliptic integral

$$\int_0^{2\pi} \sqrt{1 - k^2 \sin^2\theta} \, d\theta = 4 \int_0^{\pi/2} \sqrt{1 - k^2 \sin^2\theta} \, d\theta,$$

so the relative mixing length is given by

$$\frac{l}{r_0} = \kappa \left[\frac{1 - k^2}{2 \int_0^{\pi/2} \sqrt{1 - k^2 \sin^2\theta} \, d\theta} + \frac{z_0}{r_0} \right] \qquad (24).$$

Neglecting the term containing the roughness length, and using 0.45 as the universal turbulence constant, this expression is shown in FIGURE 1 as curve A. The agreement with observation is seen to be satisfactory.

THEORETICAL EXPRESSION FOR THE VELOCITY DEFECT IN A CIRCULAR CYLINDER

In finding the velocity defect the roughness length will be neglected. Consequently the shear becomes infinite at the wall and the result will not be applicable close to the wall. Combining equations (10) and (24),

$$\frac{u_m - u}{u_{*0}} \kappa = 2 \int_0^k \int_0^{\pi/2} \frac{\sqrt{k} \sqrt{1 - k^2 \sin^2\theta}}{1 - k^2} \, d\theta \, dk \qquad (25).$$

The mathematical function on the right side can be evaluated by the use of two expansions in series,

$$1/(1 - k^2) = 1 + k^2 + k^4 + \ldots \qquad (k^2 < 1),$$

$$\int_0^{\pi/2} \sqrt{1 - k^2 \sin^2\theta} \, d\theta$$

$$= \frac{\pi}{2} \left[1 - \left(\frac{1}{2}\right)^2 k^2 - \left(\frac{1 \times 3}{2 \times 4}\right)^2 \frac{k^4}{3} - \left(\frac{1 \times 3 \times 5}{2 \times 4 \times 6}\right)^2 \frac{k^6}{5} - \ldots \right] \qquad (k^2 \leq 1),$$

so it becomes

$$\pi\int_0^k \sqrt{k}\left\{1+\left[1-\left(\frac{1}{2}\right)^2\right]k^2+\left[1-\left(\frac{1}{2}\right)^2-\left(\frac{1\times 3}{2\times 4}\right)^2\frac{1}{3}\right]k^4+\ldots\right\}\mathrm{d}k.$$

On integration the result may be written

$$2\int_0^k\int_0^{\pi/2}\frac{\sqrt{k}\,\sqrt{1-k^2\sin^2\theta}}{1-k^2}\,\mathrm{d}\theta\,\mathrm{d}k=\frac{2}{3}\pi k^{3/2}\sum_{n=0}^{\infty}a_n k^{2n} \qquad (26a),$$

$$a_n=\frac{3}{4n+3}\left[1-\left(\frac{1}{2}\right)^2-\left(\frac{1\times 3}{2\times 4}\right)^2\frac{1}{3}-\ldots-\left(\frac{1\times 3\times 5\times\ldots\times(2n-1)}{2\times 4\times 6\times\ldots\times(2n)}\right)^2\frac{1}{2n-1}\right] \qquad (26b).$$

Some values for this function are given in TABLE 1.

According to equation (26a) the shear vanishes at the axis, a property lacking in both the previous theories. The ratio $(u_m - u)/u_{*0}$ according to equation (25) is drawn as curve A in FIGURE 2, using 0.45 for the universal turbulence constant. The agreement with the observations is satisfactory, and is definitely better than for curve B or curve C.

TABLE 1. VALUES OF THE FUNCTION

$$2\int_0^k\int_0^{\pi/2}\frac{\sqrt{k}\,\sqrt{1-k^2\sin^2\theta}}{1-k^2}\,\mathrm{d}\theta\,\mathrm{d}k$$

k	Function	k	Function
.00	0.000	.50	0.811
.02	0.006	.55	0.957
.04	0.017		
.05	0.023	.60	1.119
		.65	1.301
.10	0.066		
.15	0.123	.70	1.509
		.75	1.749
.20	0.190		
.25	0.267	.80	2.036
		.85	2.395
.30	0.355		
.35	0.452	.90	2.880
		.93	3.290
.40	0.560		
.45	0.679		

DETERMINATION OF THE UNIVERSAL TURBULENCE CONSTANT

In arriving at a suitable value for the universal turbulence constant, principal consideration is given to the ratio $(u_m - u)/u_{*0}$ at a relative

distance from axis of 0.9. This point is chosen far from the axis, the origin through which the curve passes automatically, and yet far enough from the wall to avoid the complications at the boundary. At this point the observed ratio as given below is 6.47 for smooth pipes, determining in comparison with the function in TABLE 1 a value for the universal turbulence constant of 0.445. For rough pipes the ratio is 6.21, determining the constant as 0.464. The average of these two is about 0.45, which was adopted in drawing curve A in FIGURE 2. This curve agrees with the observations sufficiently well throughout its length, so the value appears satisfactory.

SELECTION OF DATA

The most comprehensive data available on the velocity distribution for flow through long circular cylinders are those of Nikuradse, the fluid being water. His first report (1932) gives the results for smooth pipes, the second report (1933) those for rough pipes. In his experiments the speed of flow was measured just outside the pipe at the exit. Consequently the measured speed "at the wall" is not zero; actually it is of the order of magnitude of half the speed at the axis. The measurements close to the wall, at relative distances from axis of 0.96 and 0.98, must therefore be considered questionable.

When the viscous stress is important at the wall, as is the case with smooth pipes or with rough pipes at low Reynolds numbers, the radius r_0 used in computing theoretically the distribution of mean velocity within the turbulent flow should apparently be the radius of the turbulent core, exclusive of the laminar boundary layer (Montgomery, 1940, p. 12). For smooth pipes Nikuradse gives velocity distributions for 16 runs, for Reynolds numbers from 4000 to 3 240 000. The relative thickness of the laminar boundary layer, computed from

$$\frac{\delta}{r_0} = 7.8 \frac{\nu}{r_0 u_{*0}} \qquad (27),$$

(Montgomery, 1940, p. 14) where δ is the thickness of the laminar layer and ν is the kinematic viscosity, ranges from 0.055 to 0.00014. In order to eliminate the difficulty in regard to the radius of the turbulent core, those runs have been chosen for which the thickness of the laminar layer may be considered negligible compared with a tenth of the radius, the latter being the distance from the wall at which the velocity is important for present purposes. A relative thickness of the laminar layer of 0.002 may be considered negligible, leaving 9 acceptable runs, namely those with a Reynolds number greater than 205 000.

The mean values of $(u_m - u)/u_{*0}$ for these 9 are given in TABLE 2, and are the points plotted in FIGURE 2. The mean values of relative mixing length for these same 9 runs are the points plotted in FIGURE 1 (Nikuradse, 1932, Zahlentafel 5).

TABLE 2. MEANS OF OBSERVATIONS OF $(u_m - u)/u_{*0}$ AS FUNCTION OF RELATIVE DISTANCE FROM AXIS IN CIRCULAR CYLINDERS

$k = \frac{r}{r_0}$	$\frac{u_m - u}{u_{*0}}$	
	Smooth[a]	Rough[b]
.00	0.00	0.00
.02	0.02	0.02
.04	0.04	0.06
.10	0.15	0.17
.20	0.42	0.44
.30	0.77	0.79
.40	1.22[c]	1.22
.50	1.79	1.77
.60	2.48	2.44
.70	3.35	3.31
.80	4.53	4.42
.85	5.37	5.16
.90	6.47	6.21
.93	7.46	7.11
.96	8.96	8.45
.98	10.55	9.94
1.00[d]	15.15	14.05

[a] Mean of the 9 runs for smooth pipes with Reynolds numbers 205 000 and greater, from Nikuradse (1932, Zahlentafel 7).

[b] Mean of (1) the 4 runs for rough pipes having relative roughness 1/507 and Reynolds numbers 186 000 and greater and (2) the 3 runs for relative roughness 1/252 and Reynolds numbers 202 000 and greater, from Nikuradse (1933, p. 18).

[c] Nikuradse's value of 1.56 at Reynolds number 3 240 000 is a misprint. The value 1.16 has been used here, as computed from the speeds and friction velocity (Nikuradse, 1932, Zahlentafel 2 & Zahlentafel 4).

[d] Note that these values are computed from the measured speed at unit relative distance from axis, measured outside the exit of the pipe.

With a rough boundary it is customary to reckon distance from the boundary as the distance from the mean elevation of the boundary. In accord with this Nikuradse's values for r_0 are the mean radii of his pipes. Similarly in equations (11) and (20) it may be assumed that z and s are so reckoned. Since there may be some question about this procedure, however, the uncertainty has been eliminated by rejecting the runs in pipes having a large *relative roughness*, the ratio of the diameter of sand grains forming the roughness elements to the radius of the

pipe. The runs were chosen from the two groups having smallest relative roughness, namely, 1/507 and 1/252. From these groups were used again only the runs with a computed relative thickness of the laminar layer of less than 0.002, leaving a total of 7. The means for these are given in TABLE 2 and in FIGURE 2.

DISCUSSION OF THE PAPER

Prof. Kurt O. Friedrichs (*New York University, New York, N. Y.*) communicated by letter previous to the conference:

It is really surprising how well Dr. Montgomery's hypothesis fits with experimental results. It would be quite natural to try similar hypotheses, as for example

$$l = \kappa \left[\frac{1}{\sqrt{\frac{2}{\pi}\int_0^{2\pi} \frac{d\theta}{s^2}}} + z_0 \right]$$

instead of equation (20), which is also consistent with relation (11). For a circular cylinder this would lead to the simpler formula

$$l = \kappa \left(\frac{1 - k^2}{2} r_0 + z_0 \right).$$

If this is fitted so as to give $l/r_0 = 0.14$ for $k = 0$, $z_0 = 0$, one obtains for the turbulence constant the value, $\kappa = 0.28$. It is thus apparent that this simpler formula represents the experimental results rather poorly in contrast to Dr. Montgomery's more involved formula.

Of course Dr. Montgomery's hypothesis is of an empirical character. It refers to the situation in the large and thus is at variance with the property of the basic laws of nature to be expressible as relations in the small. The similarity hypothesis of von Kármán, on the other hand, was so devised as to have that property. However, this need not be an objection. It is not unlikely that the stability of unsteady flow depends materially on the situation in the large and that accordingly turbulent flow cannot be characterized by conditions in the small.

Dr. William F. Whitmore (*Naval Ordnance Laboratory, Navy Yard, Washington, D. C.*):

The question of the exact form of average employed in getting an average distance to be used in the expression for l might be given more consideration. An arithmetic average is unsatisfactory, as mentioned, but there are innumerable averages possible in addition to the one used. One which comes to mind immediately is

$$1 \Big/ \sqrt{\int_0^{2\pi} \frac{d\theta}{s^2}}.$$

(Note communicated later.—Subsequent calculation shows the elliptic integral to be replaced by a constant, giving a parabolic law which Dr. Montgomery informs me is in disagreement with the observations, so this average is not so desirable—there is still need for a final decision on the best average, however.)

It is possible, of course, that the resulting curve is not very sensitive to such changes, but quite possibly the value of κ might be altered. Ideally, of course, the form of average used should be determined by general considerations of hydrodynamical theory, but that is perhaps too much to expect at present.

Prof. A. F. Spilhaus (*New York University, New York, N. Y.*):

The average used in the expression for mixing length is taken over a plane angle; might it not be further generalized so as to be taken over a solid angle?

Prof. B. Haurwitz (*Massachusetts Institute of Technology, Cambridge, Mass.*):

Professor Spilhaus remarks that the integral from which the mixing length is obtained is extended only around the circumference of the cylinder and that it would

seem more appropriate to extend the integral over the whole cylinder surface. This remark leads to the question whether the theory can be extended to flow in non-cylindrical vessels or around obstacles.

Reply by R. B. Montgomery:

The problem considered here is strictly one of two-dimensional flow such that the direction of the mean velocity is everywhere parallel to a cylindrical boundary and that its magnitude varies only in a plane normal to the cylinder. The model is of infinite extent, so the turbulence is fully developed. This has the consequence, apparently, that an expression for mixing length can be set up which involves only the geometry of the boundary. The expression contains an average, and this problem can be characterized completely by an average limited to a plane. In suggesting a solid-angle average Prof. Spilhaus implies that the resulting expression for mixing length might apply to other problems, as for example to turbulent flow past a sphere. If some modification of the concept of mixing length should be useful in these other problems, it would not seem reasonable that mixing length would depend only on the geometry of the boundary—mixing length would presumably not be constant on a concentric shell about a sphere. The approach seems inherently limited to the problem specified, and generalization to include other problems does not appear possible.

Prof. B. Haurwitz:

Though the more accurate determination of κ is certainly important and useful, the foremost merit of Dr. Montgomery's paper seems to lie in the fact that it links up the case of flow over a plate, that is, a cylinder of infinite radius, and over a cylinder.

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Contribution No. 293

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Vol. 21, No. 1, February, 1943

THE INFLUENCE OF TEMPERATURE UPON THE OXYGEN CONSUMPTION OF THE CUNNER (TAUTOGOLABRUS ADSPERSUS WALBAUM) IN SUMMER AND IN WINTER [1]

NIELS HAUGAARD AND LAURENCE IRVING
Woods Hole Oceanographic Institution, Massachusetts, and Edward Martin Biological Laboratory, Swarthmore College, Pennsylvania

TWO FIGURES

It is well known that the oxygen consumption of cold blooded animals increases with temperature until a harmful temperature is reached and there is a decrease in metabolism. The relation between temperature and metabolism differs among the species of cold-blooded animals. Benedict ('32) found that the metabolism of several species of reptiles reacted very differently to changes in temperature. Studies on the metabolism of fish also show that the effect of temperature is not alike in all species. In gold fish and the Pacific killifish Fundulus parvipinnis, the relation between temperature and metabolism is almost identical (Ege and Krogh, '16; Wells, '35 b), while trout are much less sensitive to temperature changes (Gardner and King, '22; Gardner, King and Powers, '22).

That the influence of temperature upon metabolism can be very different in the same, or nearly related, species of cold-blooded animals at different geographical locations has been shown by Spärck ('36). He compared the oxygen consumption of several marine mollusks (lamellibranchs) at different latitudes, and found that the Arctic forms had a much higher rate of oxygen consumption than the temperate and Mediterranean forms.

That quite different temperature metabolism curves can be expected in summer and in winter for the same species of animals follows from experiments on acclimatization, such as those of Wells on teleost fish ('35 a). It was found that fish which had been kept in the laboratory at a low temperature had a higher rate of metabolism at an intermediate temperature than fish which had been kept at a higher temperature.

There are some direct observations to show that metabolic rates show seasonal differences. Cronheim ('11) concluded from an analysis of Knauthe's (1898) experiments that the oxygen consumption of the carp

[1] Contribution No. 293 Woods Hole Oceanographic Institution.

is dependent upon the time of the year. The metabolism was found to be higher in the spring than in the fall. Wells has studied the influence of season on the metabolic rate of the Pacific killifish Fundulus parvipinnis ('35 c). He found a seasonal change in metabolism in fish kept in water at low and at high temperature, as well as in fish kept in water which changed with the seasons.

Edwards and Irving ('43 b) found that the metabolic rate of the sand crab Emerita talpoida Say was different in summer and in winter. The animals were kept at the seasonal temperature of the sea water, and their oxygen consumption was determined over a wide range of temperatures. It was found that the winter animals had a higher rate of oxygen consumption at all temperatures below 17°–20° C. It seemed that the increased metabolic rate at low temperatures in winter offset the depressant effect which cold would have had upon the summer rate of metabolism, for these Emerita remained active and grew throughout the year. Talorchestia megalophthalma, on the other hand, showed no change in winter metabolic rate, and spent the winter in practical hibernation (Edwards and Irving, '43 a).

This short review of the literature shows that the relation between temperature and metabolic rate in cold-blooded animals may vary from species to species, and that the metabolic rate is dependent upon the environment from which the animal has been taken. In the experiments described in this paper, it has been the aim to compare the relation of metabolism of the cunner Tautogolabrus adspersus Walbaum to temperature in summer and in winter. The fish were all taken fresh from a single natural situation, and their oxygen consumption has been determined over a wide range of temperatures. The method used in the determination of oxygen consumption allowed measurements to be continued for periods of time long enough to give consistent results without damage to the fish.

ANIMALS AND METHODS

The fish were kept in tanks with running water at the temperature of the sea at Woods Hole. In summer the temperature was 18°–22° C.; in winter 1°–3°. The fish were not fed during their stay in the tank and were kept in the laboratory less than a week. In most cases their stay lasted only a few days. In summer the animals were caught along the shores at Woods Hole and brought fresh to the laboratory. Since cunners are not found along shore in winter time the animals for winter use were caught in October and kept in a live car until late December.

Fish weighing from 25 to 70 gm. were found very suitable for respiration studies. They were quiet and moved only occasionally in the respiration chamber. Each fish was used in only one experiment, in which the oxygen consumption was determined at one temperature, or, in a few cases, at two or three different temperatures. At the end of the experiments the fish were dried off with paper towels and weighed. The figures for oxygen consumption are given on the basis of wet weight.

The oxygen consumption was determined by a volumetric respirometer described elsewhere (Scholander, Haugaard and Irving, '43). It consists of a closed system of water and air of constant volume, in which the water is kept in equilibrium with the air. The carbon dioxide produced by the fish is absorbed in the gas phase, and the oxygen used is replaced from a syringe, so that the volume of the gas phase is kept constant. The pressure of the air in the respirometer is balanced against a compensating vessel to obviate the effects of temperature on the volumetric measurements. The oxygen added is measured by reading on a scale the position of the plunger of the syringe containing the oxygen supply. The readings can in this way be made as frequently as desired. During experiments the respirometer was submerged in a water bath with temperature constant to $\pm 0.1°$.

The respirometer was tested in several ways. By analysis it was found that the water was in equilibrium with the gas phase of the system. However, the oxygen content of the water decreased approximately 0.1 cc. per liter per 10 cc. oxygen consumption of the fish, because the oxygen added to replace that which was consumed contained 3–4% nitrogen. The change in volume of the system which resulted from this decrease in oxygen tension has been shown to be negligible. The sea water used in the experiments consumed approximately 0.1 cc. oxygen per hour. This amount has been subtracted from the figures for oxygen consumption.

The advantages in using this scheme are that the oxygen tension of the water in the respirometer is kept approximately constant and that the oxygen consumption of the individual fish is continuously followed and measured at frequent intervals, without summation of the errors which may arise in making single readings.

EXPERIMENTS

Figure 1 shows some typical records of individual experiments. The amount of oxygen in the syringe was recorded every 10 minutes, so that the slopes of the curves give the oxygen consumption of the fish.

In calculating the rate of oxygen consumption, the figures are converted into cubic centimeters of oxygen at zero degrees Centigrade. The blank is indicated for comparison. The records show the regularity of the measurements. In most cases the oxygen consumption was higher in the beginning of an experiment, but a steady rate was reached within 10 to 30 minutes. The steady rate was found in all cases except at extreme temperatures, which evidently injured the fish. At ordinary temperatures the steady level was maintained for many hours, and there was no sign that the rate of metabolism changed in experiments lasting

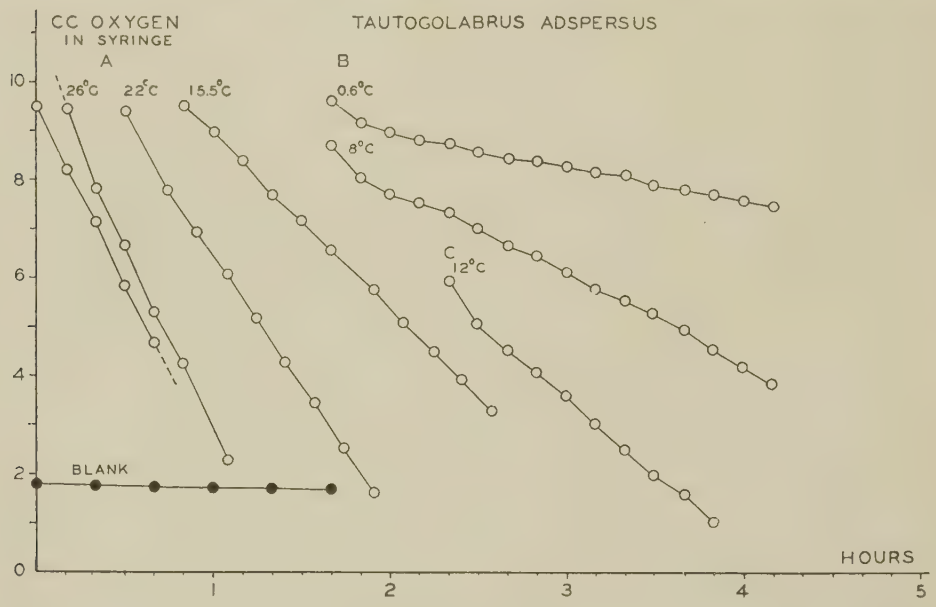

Fig. 1 Typical records of the oxygen consumption of the cunner (Tautogolabrus adspersus). The slope of the curves gives the oxygen consumption. A. A fish in summer at three different temperatures. B. A "winter fish" at two different temperatures. C. A "winter fish" at 12°C.

up to 10 hours. Since the fish were very quiet in the respiration chamber at all temperatures, the differences in rate of oxygen consumption could only in a small degree be caused by differences in activity.

Three experiments were made to investigate the influence of reversal of the temperature change. The oxygen consumption was first measured at one temperature. The respirometer was next transferred to a water bath at a different temperature until steady measurements were obtained. The respirometer was then returned to the water bath at the initial temperature. The results of the experiments are given in table 1. It is seen that the fish returned to its original oxygen consumption

TABLE 1

Oxygen consumption in cubic millimeters per gram per minute as the temperature was changed and restored.

WEIGHT, GRAMS	26°C.	21°C.	26°C.
50	3.1	2.1	3.1
49	3.4	1.8	3.4
30	2.9	2.1	2.9

after having been subjected to a change in temperature. These experiments and the fact that a steady rate of oxygen consumption was obtained at all temperatures for individual fish indicated that a general picture of the temperature-metabolism relation of the cunner fresh from natural conditions could be obtained by the determination of the rate of oxygen consumption of a number of fish over a wide range of temperatures.

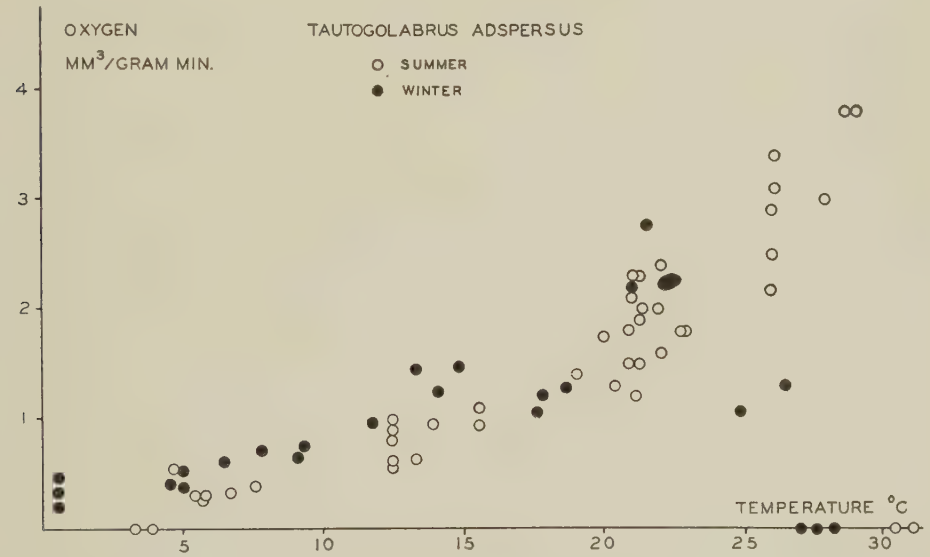

Fig. 2 The influence of temperature on the oxygen consumption of the cunner (Tautogolabrus adspersus) in summer and in winter. Each point is an average of from 1 to 4 hours of steady rate of oxygen consumption. The fish weighed from 25 to 70 gm.

Figure 2 shows the results of such experiments. Each point represents the average of from 1 to 4 hours of steady rate of oxygen consumption, as exemplified in figure 1. The experiments in summer were done in August and September, while the winter experiments were done in late December and the beginning of January. From the figure it can be seen that the rate of oxygen consumption of the "winter

fish'' was a little higher than that of the ''summer fish'' at all temperatures below 15°. At 5°, which is near the natural temperature for the cunner in winter, the oxygen consumption was about one-fourth of the oxygen consumption of the ''summer fish'' at 20°–21°. The ''summer fish,'' however, decreased its metabolism to about one-fifth of its normal value by being subjected to a temperature of 5°. There seems, therefore, to be a small difference between the metabolism of the cunner in summer and in winter.

The ability to withstand high and low temperature was also different in summer and in winter. In summer the fish could not survive transfer to water at 30°, while steady records of the oxygen consumption at 28°–29° have been obtained. Five degrees was the lowest temperature the ''summer fish'' could endure. When transferred to water at this temperature the fish lost their sense of balance, and all fish subjected to lower temperatures died quickly. In the case of the ''winter fish,'' it was possible to measure the oxygen consumption at 25°–26°, but all fish subjected to higher temperatures died. A steady rate of oxygen consumption was still found at 0.6° (fig. 1 B), which was the lowest temperature tried.

DISCUSSION

Since the cunner in winter has a slightly higher rate of oxygen consumption than in summer at temperatures below 15°, a certain amount of acclimatization has taken place. However, the oxygen consumption in winter at the natural winter temperature of the sea water is only about one-fourth of the metabolism of the ''summer fish'' at its natural environmental temperature, and the rate of metabolism in winter falls far short of that which is usual in summer. The observations of the lethal temperature show that there is a somewhat better ability to withstand high temperature in summer, but that this difference is small.

It is interesting to compare the information given by the experiments with our knowledge of the natural habits of cunners. They are found in abundance along the shores at Woods Hole in the summer months June to September. Outside this period, they are, however, rarely found. In winter, cunners are not found along the shores, and they may spend the season in deeper waters near by, since there are no records of their actual migration. In the Gulf of Maine, cunners are caught in winter at the same places where they are found in summer (Bigelow and Welsh, '24), but in these situations the winter temperature may not be as low as it is along shore at Woods Hole. Some authors claim that cunners pass the winter in the mud. This may be

correct in some cases, for Mr. A. M. Hilton of the Supply Department of the Marine Biological Laboratory informed us that, when the new bridge was being built at Woods Hole in the winter of 1940, many cunners were dredged up with the mud from a deep hole. It was also observed that the cunners held in captivity were much more active in summer than in winter. In summer the animals swam actively, but in winter they were very quiet and stayed near the wall of the live car.

The information about the winter habits of the cunner seems to agree with the experimental observations. With the low metabolic rate brought about by winter temperature, the cunners apparently can not remain in their usual summer habitat. Their disappearance appears to be an acknowledgment of their inability to make suitable adjustment of metabolism to winter conditions.

SUMMARY

The oxygen consumption of the common cunner (Tautogolabrus adspersus Walbaum) has been determined in summer and in winter at temperatures ranging from 0° to 30°.

Below 15° the oxygen consumption was found to be a little higher at each temperature in winter than in summer. Since the difference is small, the depression of metabolism caused by low temperatures in winter probably leaves too little physiological activity to enable the cunners to stay in their summer habitat. This view agrees with the disappearance of the cunner from the shore line waters in winter.

ACKNOWLEDGMENT

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Contribution No. 298

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A Volumetric Respirometer for Aquatic Animals*[1]

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(Received January 13, 1943)

A closed circuit respirometer is described which circulates a given volume of water through the chamber containing the fish and equilibrates the water with a small volume of air. As oxygen is consumed by the fish, its removal from the air space is indicated manometrically, and is compensated for by measuring in oxygen from a syringe. Continuous, direct measurements can be made at any intervals and for many hours. The instrument described was used for cunners weighing from 30–70 grams and for toadfish weighing 300 grams, with oxygen consumption from 1 to 12 milliliters per hour. It showed accurately the use of $\frac{1}{10}$ milliliter oxygen per hour.

THE method presented permits the determination of the oxygen consumed by a fish or other aquatic animal by direct volumetric measurement. The measurement is continuously carried on at constant tension of oxygen in a closed system and thus no summation of errors takes place in the calculation of the results. The accuracy of the present machine is about $\frac{1}{10}$ milliliter per hour.

PRINCIPLE

The apparatus is a closed system of constant volume in which water is continually circulated and kept in equilibrium with a gas phase of the system. The carbon dioxide produced by the animal is absorbed in the gas phase and the oxygen used is accurately replaced from a syringe so as to keep the volume of the gas phase constant. In order to obviate errors from fluctuations of barometric pressure and temperature, the pressure of the gas phase is balanced against a compensating vessel.

DESCRIPTION OF APPARATUS

The apparatus (see Fig. 1) consists of a fish chamber (*A*) shaped so that it just accommodates the fish. The chamber is closed by means of the screw bolts indicated in the figure. Between the top flange of the chamber and the lid is placed a rubber gasket so that the chamber is watertight. The chamber is connected with a simple centrifugal pump (*B*) preferably of stainless steel. The pump, which is driven by a constant-speed electric motor, carries the water through a suction pump (*C*) into the equilibration vessel (*D*). The suction pump mixes the water with air, and air bubbles are carried down into the equilibration vessel. The water is thus kept in equilibrium with the gas phase in the system. The bubbles are checked from entering the fish chamber by a baffle placed in *D*. The bottom of *D* is connected back to the fish chamber. The outlet of the fish chamber should be placed so as to allow air bubbles collecting in the chamber to escape.

The air which is circulated by the suction pump passes from the equilibration vessel around a baffle and through a water-drop trap into a U-tube (*E*) containing soda lime or ascarite,[2]

* Contribution No. 298 from the Woods Hole Oceanographic Institution.

[1] The glassware was made by J. D. Graham. The apparatus was demonstrated at the Marine Biological Laboratory at Woods Hole, August 27, 1941.

[2] A commercial carbon dioxide absorbent manufactured by Arthur H. Thomas Company, Philadelphia.

back to the suction pump and down into *D* again. The air circuit is open to a manometer (*F*) which is connected to the compensating vessel (*G*). Oxygen is stored in a 10-cc syringe (*H*) which is connected to the circuit through capillary tubing. The connection may conveniently be made as a ground glass joint (*I*) which provides a water trap to prevent mixing of the oxygen in the syringe with the air in the circuit. The syringe is conveniently read by fastening an indicator to the plunger and a scale to the mounting board.

The whole apparatus is mounted on a piece of wood or plastic and immersed in a large water bath at constant temperature. It is placed so deep that the main gas and water volumes are below the water surface of the bath. In the apparatus used, the gas phase in the circuit was 40–50 cubic centimeters and the amount of water about 1 liter.

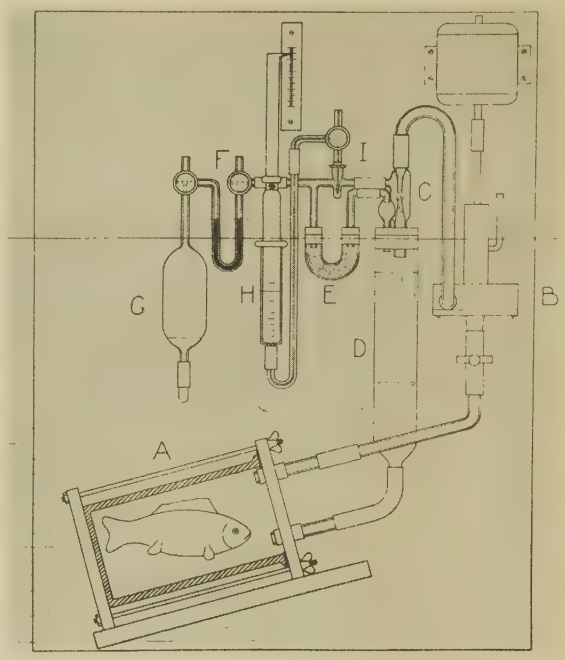

FIG. 1. Diagram of the respirometer. *A*, chamber for fish; *B*, centrifugal pump for circulation of water; *C*, aspirator; *D*, equilibration chamber; *E*, tube containing absorber for carbon dioxide; *F*, manometer; *G*, compensating vessel; *H*, syringe for injection of oxygen; *I*, stopcock for addition of new oxygen supply and leading oxygen to the circuit.

SETTING THE APPARATUS

Soda lime, or ascarite, is put into the absorption tube, a little water is placed in the compensating vessel, and the manometer is filled with Brodie solution or a 1 percent solution of Tergitol.[3]

The fish chamber is filled with water and connected with the apparatus. The equilibration vessel is filled almost to the top with water. Leaving both stopcocks (*F*) open, the equilibration vessel is pushed tight against its stopper. Oxygen is drawn into the syringe from a rubber bag and the stopcock (*I*) turned as in the Fig. 1. The motor driving the pump is started. The amount of bubbling is adjusted by regulating the pump intake by means of the screw clamp indicated in the figure. The apparatus is placed in the water bath.

The air circuit is disconnected at the suction pump and the apparatus run while open to air until temperature equilibrium has been obtained and the water is in equilibrium with the outside air. This will take from 10 to 30 minutes, depending upon the temperature of the water bath. The air circuit is now connected and the stopcocks closed to the outside air.

[3] A commercial wetting solution manufactured by Carbide and Carbon Chemicals Corporation, New York.

If tap water is used there should be no oxygen consumption after 5 to 10 minutes; that is, no oxygen should need to be injected from the syringe to keep the manometer balanced. Sea water, unlike tap water, gives a small blank, which is probably caused by micro-organisms in the water.

PROCEDURE

Taking care to handle it under water, a fish is quickly put into the chamber. The chamber is connected with the apparatus and equilibrated in the water bath with the air circuit disconnected at the suction pump, as described above. This assures temperature equilibrium with the water bath and equilibrium with the outside air before the measurements begin. The stopcocks are then closed.

As oxygen is consumed, the volume of the gas phase diminishes, and the manometer fluid is pulled over towards the circuit. This movement is accurately compensated for by injecting oxygen

TABLE I.

Concentration of oxygen		Ratio of oxygen in water to oxygen in air
Gas phase %	Water cc/liter	
21.0	5.60	0.267
21.5	6.00	0.279
23.0	6.30	0.274
26.0	7.15	0.275
28.0	7.70	0.275

The analyses were done 5 minutes after the concentration of oxygen had been changed.

from the syringe at regular intervals so that the manometer menisci are kept leveled. The syringe indicator is read at convenient intervals; it gives the oxygen consumed directly in cubic centimeters, or other units, at the temperature of the water bath and at atmospheric pressure. When the oxygen supply is used up, new oxygen is drawn into the syringe through the three-way stopcock without disturbance of the experiment.

TESTING THE APPARATUS

There are two main conditions which require special attention in order to insure that the measurement of oxygen consumption made in the gas phase is equal to the oxygen taken up from the water: (1) There must always be equilibrium between the gas phase and the water in the apparatus, and (2) the oxygen tension in the apparatus must remain sufficiently constant during an experiment so that no significant volume change of the gas phase occurs.

To determine the equilibration of the gas phase and water, the following experiment was done. During a blank run, the oxygen concentration was determined in the gas phase and in the water. The oxygen tension was then considerably altered by replacing some of the air with pure oxygen. Five minutes after each replacement samples of water and gas were taken for analysis. The oxygen in the air was determined in about 30 cubic millimeters by a micro method developed by Scholander and Roughton.[4] The oxygen content in the water was determined by Krogh's[5] modification of Winkler's method in a sample of 2 cubic centimeters. The result of the experiment is seen in Table I. Since the relation between the concentration of oxygen in the gas phase and in the water is constant, it is indicated that equilibrium was reached in all cases.

That the oxygen tension remained sufficiently constant was determined during actual experiments with a fish in the machine. Samples were taken from the gas phase and the water, and analyzed as above. During a 5-hour experiment with a fish in the machine, the oxygen content of the water was usually found to decrease 0.1 cubic centimeter per liter per 10 cubic centimeters oxygen consumption of the fish. This decrease resulted from the fact that the added oxygen was not pure, but contained 3–4 percent nitrogen. Some of the oxygen used by the fish was thus replaced by nitrogen.

Blank experiments were then conducted to determine the volume change of the gas phase for a given change in oxygen tension. One cubic centimeter oxygen was injected in the gas phase, letting out 1 cubic centimeter air at the same time. According to calculations, this corresponded to an increase in the oxygen in the water of 0.5 cubic centimeters per liter and resulted in a volume decrease of 0.1 cubic centimeter. In actual experiments a fish did not lower the oxygen tension in the water more than 0.1 cubic centimeter per liter per 10 cubic centimeters oxygen consumption. The volumetric error due to the drop in oxygen tension was, therefore, less than 1 percent of the oxygen consumption.

In some experiments the water was tested with cresol red as an indicator to see if carbon dioxide accumulated in the water. No accumulation was found with a good rate of circulation. Accumulation took place, however, if the circulation were much diminished.

The machine was often run without fish for blank determinations. For sea water a blank was found amounting to about $\frac{1}{10}$ cubic centimeter per hour, which is probably caused by microorganisms in the water. Blanks on the sea water used in an experiment, after the fish had been taken out, were found to be of the same order of magnitude as blanks on fresh sea water.

Figure 2 shows two typical records of the

[4] P. F. Scholander and F. J. W. Roughton, J. Ind. Hygiene and Toxicology 24, 218 (1942).
[5] A. Krogh, Ind. and Eng. Chem. An. Ed. 7, 131 (1935).

oxygen consumption of cunner (*Tautogolabrus adspersus*) at two different temperatures. The blank is indicated for comparison. The method does not appear to have any harmful effect on the fish. Experiments were often continued over long periods of time, up to 11 hours, with perfectly steady oxygen consumption and the fish seemed in perfect condition when taken out.

The respirometer has been used for cunner, weighing from 30 to 70 grams, and for toadfish, *Opsanus tau*, weighing up to 300 grams, with oxygen consumption varying from 1 to 12 cubic centimeters per hour. For larger and smaller animals the dimensions of the apparatus can probably be considerably changed without alteration of design.

The research was supported in part by a grant to Laurence Irving from the American Philosophical Society.

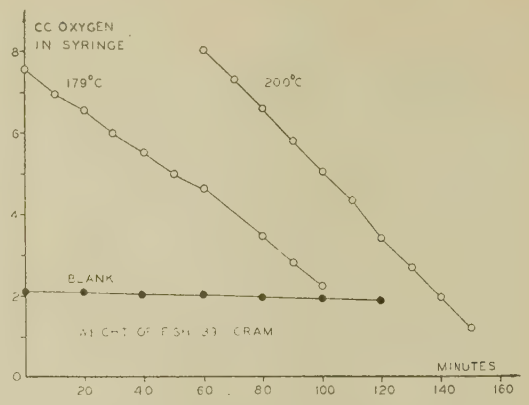

FIG. 2. Oxygen consumption of *Tautogolabrus adspersus* at two different temperatures.

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THE INFLUENCE OF TEMPERATURE AND SEASON UPON THE OXYGEN CONSUMPTION OF THE SAND CRAB, EMERITA TALPOIDA SAY [1]

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FOUR FIGURES

The metabolic rate of poilikothermous animals changes directly with temperature, and their activity is thus dependent upon the temperature fluctuations of the environment. As an apparent consequence of the depressant effect of cold some poikilotherms become quite inactive in winter. Others remain active and for them the effect of temperature is apparently small. Various species may differ considerably with respect to the effect of temperature upon their metabolism, as was shown in the review of Krogh ('16) and in numerous subsequent studies.

There are indications that the effect of temperature may vary with season, and internal changes in the animal may thus offset the environmental changes in temperature in the direction of preserving stable metabolism through the seasons. Wells ('35 a) found that the metabolism of the Pacific killifish was higher at 15° in February and March than at the same temperature in July and August. In the cunner the oxygen consumption in winter was a little greater at temperatures below 15° C. than in summer at the same temperatures (Haugaard and Irving, '43). Wells ('35 b) found that fishes previously exposed in the laboratory to a high temperature had a lower metabolism at an intermediate temperature than was shown by fishes previously exposed to a low temperature.

Further indication for the adjustment of metabolism to a prevailing temperature can be derived from the differences in the metabolism of related poikilothermous species from different geographical regions. At a given temperature, certain species and varieties of marine lamellibranchs from Greenland and Denmark had a higher oxygen consumption than those from the Mediterranean (Spärck, '36). The oxygen consumption of several crustaceans from Plymouth, England, was re-

[1] Contribution no. 302 from the Woods Hole Oceanographic Institution.

ported to be higher than that of the corresponding species from the colder waters of Kristinberg, Sweden (Fox, '36).

In the following study the metabolic rate of the sand crab, Emerita talpoida Say, has been determined in summer and winter over the full range of temperatures which the animals would endure. It appears that in winter the metabolic rate is accelerated at low temperatures, and these animals provide an example of an internal adjustment of the poikilothermous metabolism to external conditions. In line with their sustained metabolic rate it can be shown that Emerita remains active and grows at low winter temperatures.

APPARATUS AND TECHNIQUE

The oxygen consumption of the animals at rest was measured by means of a volumetric micro-respirometer (Scholander and Edwards, '42). This technique was adopted because of the following significant advantages not present in other micro-respirometric techniques. (a) The oxygen used by the animal is replaced every few minutes with oxygen so that the oxygen tension is kept constant. It is thus possible to measure respiration at normal oxygen tension for many hours even at high rates of oxygen consumption. By the procedure of adding oxygen the volume of gas in the system can be kept small. This largely increases the stability of the apparatus and increases the sensitivity because it renders the measured volume changes large compared with the total volume of the apparatus. (b) For the equilibration of the water, rocking of the animal chamber has been avoided by using a propeller which secures good diffusion by agitating the surface but causes the least possible disturbance to the animal. It has been determined that in this way a large diffusion of oxygen into the aqueous phase can occur despite the smallness of the animal chamber. The apparatus shares with other volumetric methods the advantage over manometric methods that direct volume readings are independent of the size of the animal chamber and the compensating vessel. The animal chamber was a shell vial of 5 cc. volume. Ascarite was used to absorb the carbon dioxide produced. The respirometers were placed in water-filled aquaria which were then placed in a large water bath with temperatures controlled to within 0.1°C.

Blanks were run on the machine at various intervals with distilled water, and with the combination of sand and sea water used, before and after actual experiments. The blank with distilled water was from 0 to 0.3 cu. mm. per hour. The blank with sand and sea water was practically constant at a given temperature and less than 1% of the

total consumption of the animal. This latter value was deducted from the oxygen consumption read on the micrometer in order to give the true oxygen consumption of the animals. All figures have been reduced to cubic millimeters of oxygen at 0°C. and 760 mm. of mercury per gram weight of animal per minute.

The range of temperatures from as close to 0 as possible up to 40°C. was investigated. Once the animals had been used at a given temperature they were then discarded for fear that the temperature effect might modify the results of subsequent experiments. At each temperature the machines were allowed 15 minutes for temperature equilibration, and the oxygen consumption was thereafter read at 15-minute intervals. The experiments usually lasted from 4 to 6 hours. When the experiment had ended the animals were removed, dried with absorbent toweling, and weighed. Drying and weighing were repeated until constant weight was obtained.

As the animals lived longest and remained quiet for from 4 to 6 hours at a stretch in the sand covered with sea water, this combination was used in the experimental vessel. The Emerita was placed in the vessel, just enough sand to cover him was poured in, and sea water was then added to cover the sand by about 1 inch. The water was then bubbled with air until the vessel was ready to attach to the respirometer. The animal usually sat still beneath the sand with just the antennules and eyestalks in view. The movement of the animal was checked by observation, and whenever a gross noticeable move was made by the animal the reading for that period was discarded, and if the movement continued the experiment was stopped.

ANIMALS

The animals used in these experiments were first year adults of the common sand crab, Emerita talpoida Say, obtained at Nobska Beach, Woods Hole, Massachusetts.

During the summer months the colony of Emerita was distributed along the entire beach in the fine-to-coarse sand of the intertidal zone, following the tides up and down the beach and seldom wandering more than a few feet from the water level. In the winter months the whole group of animals took to deeper water, and the colony split up into three isolated communities. The two colonies of first year adults were found several hundred yards from shore under 6 feet of water, and the second year adults were still farther out, under 12 feet of water. It is interesting to note that no animals were found on a stony or muddy

bottom, but one colony of second year adults was found in winter in the sand just within the sand-rock line under 14 feet of water.

The life span of Emerita at Woods Hole is a little over 2 years. The eggs are fertilized and carried by the female during the months of June and July. Early in July, a few Zöea were found, but the majority of them are found in the tow in the month of August (Bumpus, 1898).

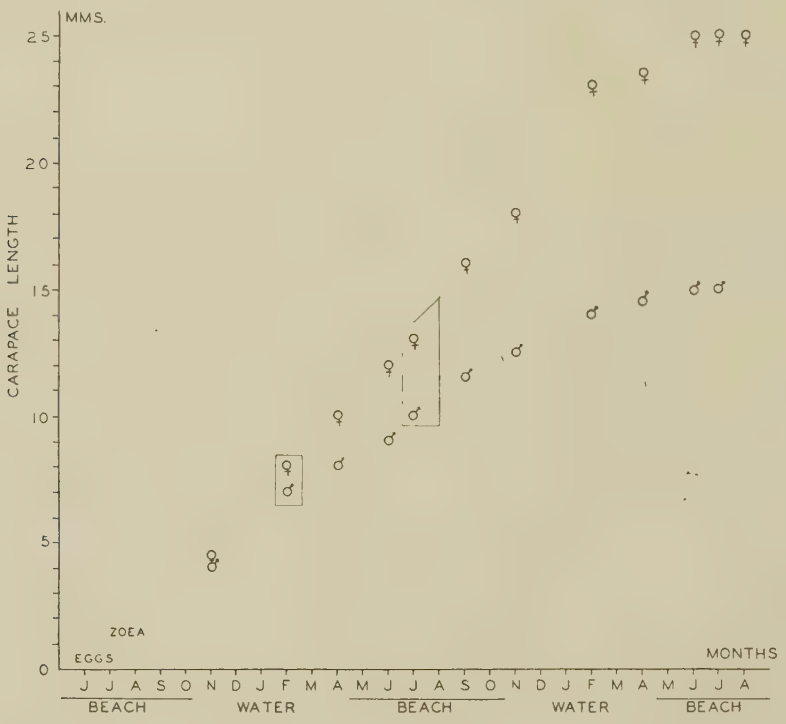

Fig. 1 Growth rate and life span of Emerita talpoida from the Woods Hole region. Each point represents the average length of one hundred animals. The points enclosed indicate those animals used in the experiments on oxygen consumption. The bottom line indicates the habitat of the animals in the different seasons.

The adult form is attained during the fall of the first year and growth seems to be steady and rapid during the winter and spring months with a gradual decline in the rate of growth during the months of the following summer (fig. 1). By this time the females have reached a carapace length on the average of from 9 to 11 mm., and the males a length about 2 mm. less than the females. During the first summer of adulthood the females carry no eggs and show little evidence of sexual activity, but the young males show a mating reaction with the older females.

Growth is somewhat slower during the second year and by May of the second year the adults have reached their final size, the females averaging 25 mm. carapace length and the males 15 mm. carapace length. During June and July of the second year the females carry eggs, and then live several months after the mating season, whereas the males die shortly after mating. Thus the life span of the male Emerita from the Woods Hole area is roughly 25 months, and that of the female about 27 months.

The animals used in the experiments to be described were taken from the first year class for two reasons: (1) because of the abundance of the animals in this class at the time the experiments were done, and (2) because of the convenience of the size of the animals for the size of the respirometers.

HYDROGRAPHIC CONDITIONS

In order to see the significance of the reactions of the animals to the temperatures artificially produced in the laboratory, some facts concerning the physical conditions of the natural habitat are presented. The temperature of the sea water at Woods Hole is highest during the months of July and August, averaging between 20 and 21°C. The average temperature of the air is only slightly higher (Herrick, 1895; Sumner, Osborn and Cole, '11; Fish, '35). The salinity is about 31.4 parts per thousand in the summer months (Fish, '25; Ketchum, '41). The currents at Woods Hole are strong and, as a result, the waters are turbulent. This fact coupled with an abundance of plankton at all times and saturation of the water with oxygen probably assured the animals of plenty of food and oxygen (Fish, '25).

During the fall, temperatures drop rapidly, the oxygen content rises sharply, but the salinity remains the same. The coldest months of the winter are January and February, reaching the low average of 0.5°C. in the water during the month of February (Sumner, Osborn and Cole, '11; Fish, '25). The phytoplankton and zooplankton are abundant (Fish, '25; Clarke and Zinn, '37; Lillick, '37). Hence again the factors governing growth, namely, food and oxygen, are favorable. The low temperature of the air, on the average of — 4.2°C., and the low temperature of the water are such that the sandy shores are often frozen and covered with ice. Hence it would be impossible for Emerita to dig in its accustomed manner in the intertidal zone in winter. It can be seen that Emerita is used to a seasonal range in temperature of 20–30°C.

ANALYSIS OF WEIGHT

All measurements of oxygen consumption in this paper are given in units based on the wet weight of the animals. Among invertebrates, the body composition is so variable as to make it desirable to have other dimensions than weight and length available, in order to compare metabolism with size. Accordingly, the water content of Emerita was determined by drying a number of animals to constant weight. The results shown in table 1 indicate that the average proportion of water was 73%, without significant variation between weights 143 to 795 mg.

TABLE 1

Water and digestible material content of Emerita talpoida Say.

WET WEIGHT IN GRAMS	DRY WEIGHT IN GRAMS	WATER %	WEIGHT OF DIGESTIBLE MATERIAL IN GRAMS	DIGESTIBLE MATERIAL %
0.143	0.034	76.2	0.016	11.2
0.302	0.084	72.2	0.041	13.6
0.369	0.110	70.2	0.046	12.5
0.370	0.094	74.6	0.044	11.9
0.383	0.091	76.2	0.040	10.4
0.383	0.112	70.8	0.044	11.5
0.387	0.096	75.2	0.044	11.4
0.410	0.122	70.2	0.054	13.2
0.457	0.141	69.1	0.064	14.0
0.480	0.126	73.8	0.058	12.1
0.513	0.151	70.6	0.059	11.5
0.525	0.137	73.9	0.066	12.6
0.592	0.150	74.7	0.071	12.0
0.761	0.219	71.2	0.089	11.7
0.795	0.202	74.6	0.081	10.2
		Mean 72.9		Mean 11.2

The dried material was then digested with trypsin for 2 days at 37°, washed carefully, and dried again. The loss of weight includes carbohydrate and fat released by digestion of the structural protein, as well as protein. The digestible material amounted to 11% of the wet weight and showed no significant alteration with growth.

Reference of oxygen consumption to body weight, to dry material, or hydrolyzable material should give the same sort of relation.

RESULTS

Oxygen consumption of summer Emerita

The work upon the summer Emerita was divided into three parts; (1) the oxygen consumption according to weight and sex, (2) the effect of sudden changes of temperature upon oxygen consumption and of

restoration to the original temperature, (3) the influence of various levels of temperature upon the oxygen consumption. The animals used in these experiments were collected during the months of July and August, and were slightly more than a year old. They weighed between 300 and 600 mg., whereas the animals used in the winter experiments weighed from 100 to 250 mg.

1. Oxygen consumption according to weight and sex. As one would expect, the oxygen consumption of the smaller animals per unit of weight was greater than that of the larger animals. In figure 2, in which the summer Emerita are divided into three classes according to weight,

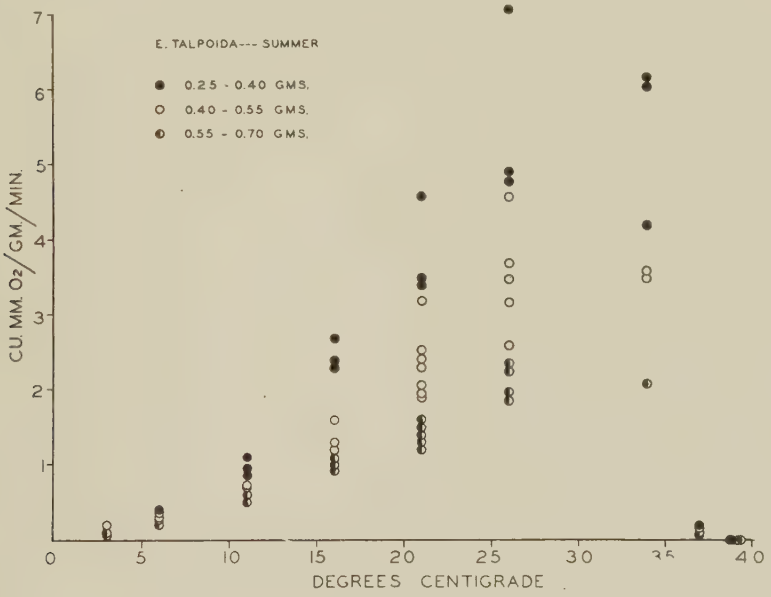

Fig. 2 The oxygen consumption of Emerita talpoida at various different temperatures during the summer. Each point represents the steady level of oxygen consumption of one animal for 4–6 hours.

it can be seen that, at a given temperature, the oxygen consumption of the smaller animals was greater than that of the larger ones. It can also be seen that the smaller animals showed a greater change in response to temperature, so that the slope of the curve representing the lowest weight group was quite steep, that of the middle weights not quite so steep and that of the higher weights was very gradual.

In figure 3, the oxygen consumption of a large number of summer Emerita is plotted against the body weight. Three different temperatures are represented. Here again it was shown that the oxygen con-

sumption of the small animals was high compared with that of the larger animals, and it is also shown that the curve relating oxygen consumption and weight was like a regular hyperbolic curve.

Few measurements of the oxygen consumption of animals of the same weight, but of different sexes, were taken. In general, the males, being much smaller than the females of the same age group, had a higher metabolism for each age.

2. Effect of sudden changes in temperature on oxygen consumption. The pulsating subumbrella ring of the scyphomedusa, Cassiopea, when taken from 29 to 36°C., assumes the rate characteristic of that temperature at once, and, when the animal is returned to 29°, it assumes the characteristic pulsation rate for that temperature once more. If, however, the temperature is raised to 37 or 38°C., the rate upon the return to 29° is slower than it was previously at that temperature (Mayer, '18). The metabolism of warm spring fishes drops when they are taken to a low temperature, and the metabolism returns to normal with return to high temperatures again (Sumner and Sargent, '40).

In our experiments, we have carried the temperatures to which the animals were exposed far beyond the natural seasonal variation. To determine the effect of sudden changes from normal to abnormal temperature and return to the normal again, experiments were done on Emerita of various sizes collected during the summer months when the temperature of the water was approximately 20°C. The results are given in table 2. When the temperature was raised from 20° to 25, 26, 28, and 29°, the oxygen consumption rose, and when the temperature was lowered again to 20° the oxygen consumption returned to the initital rate at once. When the temperature was raised to 32 and 34°, the effect upon the animal was of a different nature, and the oxygen consumption upon the return to 20° was lower than the normal at that temperature. When the animals were taken from 20° down to 15, 14, and 9°, the oxygen consumption decreased but returned to the original when the animals were returned to 20°. In one instance the lowering of the temperature to 7, and in two cases to 6°, gave the same result, but, in the other cases of lowering the temperature to 8° and below, there was a change in the animals' ability to return immediately to the normal oxygen consumption, when returned to 20° again.

These results indicate that Emerita talpoida is rapidly influenced by rapid changes from normal temperature, and as rapidly returns to the initial condition if the change is not greater than 10°C.

3. Influence of various levels of temperature upon the oxygen consumption. Determinations of oxygen consumption were made at approximately every five degrees from 3°C. upwards. Figure 2 shows the oxygen consumption of the summer Emerita talpoida at the various temperatures. It can be seen that the oxygen consumption increased with increasing temperature up to 26°, and then decreased with further increases in temperature, until at 40° the animals died almost immediately they were placed in the experimental vessel. The summer animals showed a gradual decrease between 26 and 34°, and then a sharp decrease between 34 and 37°. Not all of the animals survived the 4-hour

TABLE 2

The effect of sudden changes in temperature upon the oxygen consumption of Emerita talpoida in summer.

ORIG. TEMP. DEGREES C.	O_2 USED MM.^{3}/GM./MIN.	NEW TEMP. DEGREES C.	O_2 USED MM.^{3}/GM./MIN.	RETURN TEMP. DEGREES C.	RETURN O_2 USED MM.^{3}/GM./MIN.
20	3.4	25	4.7	20	3.4
20	1.2	26	1.8	20	1.2
20	1.9	26	3.2	20	1.9
20	2.0	26	3.1	20	2.0
20	1.2	26	1.8	20	1.2
20	2.9	28	5.7	20	3.0
20	2.5	29	3.7	20	2.4
20	2.4	32	3.6	20	1.8
20	2.3	34	3.6	20	1.8
20	3.2	15	1.7	20	3.2
20	1.3	15	0.7	20	1.3
20	1.3	14	0.7	20	1.3
20	1.4	9	0.5	20	1.3
20	1.6	8	0.7	20	0.6
20	1.3	7	0.4	20	1.3
20	2.1	7	0.3	20	1.5
20	0.9	6	0.3	20	1.0
20	0.9	6	0.2	20	1.1
20	1.8	3	0.1	20	0.4
20	1.8	1	0.09	20	0.3

exposure to 37°, and all of the animals died within a short time after the end of the experimental run at that temperature. The oxygen consumption at 37° was almost too small to measure and was of the same order as that at 3°. The movements made by the animals at all temperatures during the experiments were slight and practically unobservable, and so it is believed that the higher oxygen consumption of the smaller animals at any one temperature and the higher oxygen consumption of all the animals at higher temperatures was not due to an increase in muscular movement.

Oxygen consumption of winter Emerita

The animals used in the winter experiments were collected during the month of February, when the temperature of the water was as low as —1.5°C. They weighed from 100 to 225 mg. The animals were spontaneously active in the waters at that temperature. In the laboratory they were kept at 3°, and they swam about and burrowed actively. Determinations of the oxygen consumption were made over the range of temperatures from 3° to 30°.

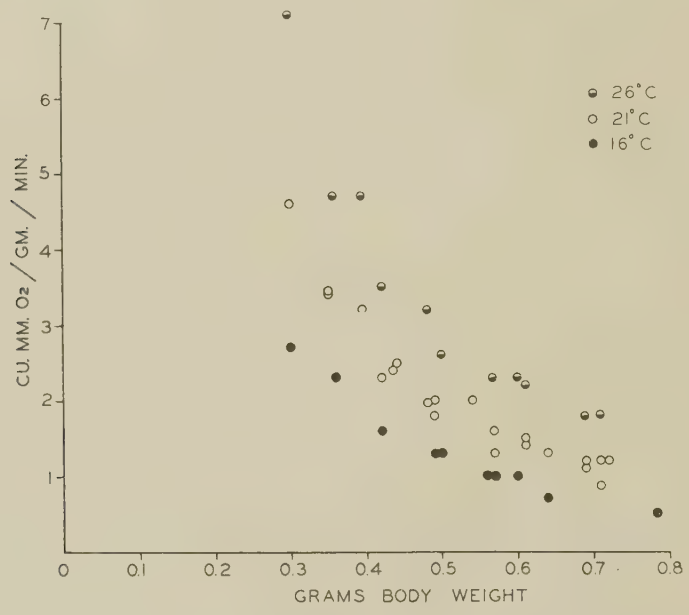

Fig. 3 The oxygen consumption of summer Emerita talpoida of various different sizes, taken at three different temperatures. Each point represents the steady level of oxygen consumption of one animal for 4–6 hours.

Figure 4 shows the oxygen consumption of the winter Emerita talpoida at the various temperatures. As in summer, the oxygen consumption increased with increases in temperature, with the difference that the maximum oxygen consumption was reached at 23°, whereas in summer the maximum was slightly higher. Beyond that temperature, the oxygen consumption dropped with a further increase in temperature, and temperatures around 27 to 30° were usually fatal to the animals. At 27°, some of the animals died during the experiment, whereas others survived the exposure to that temperature, and recovered when replaced to a lower temperature. The fall in oxygen consumption at

the higher temperatures was rather more abrupt in the winter animals than in the summer animals.

At the temperatures which are high for winter the oxygen consumption of the winter animals was greater than that of the summer animals. The difference may be expressed by saying that the oxygen consumption of the winter animals at 12° was about the same as that of the smallest of the summer animals at 17°.

At the lowest temperature, i.e., 3°C., the oxygen consumption of the winter animals was much higher than that of the smallest summer

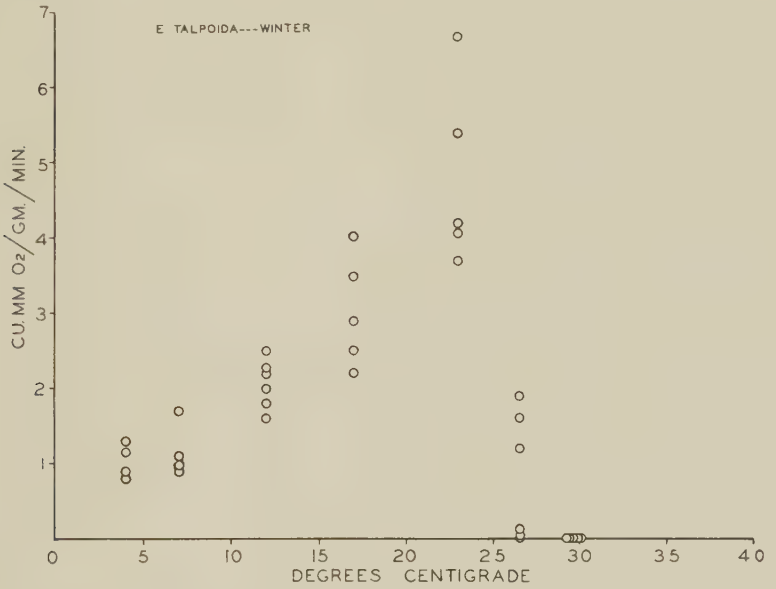

Fig. 4 The oxygen consumption of Emerita talpoida at various different temperatures during the winter. Each point represents the steady level of oxygen consumption of one animal for 4–6 hours.

animals at the corresponding temperature. The oxygen consumption of the winter animals at 3° was like that of the summer animals at 13°. The animals used in the winter months were smaller in size than those used during the summer, and hence a higher metabolism on the basis of size might be expected. However, reference to figures 2 and 4 show that at the lowest temperature the oxygen consumption of the largest winter animals was five to six times that of the smallest class of summer animals at that same temperature. This is a greater increase than could be expected on the basis of size alone, i.e., from the curves in figure 3, one could expect the winter rate of metabolism to be double that in summer because of the smaller size of the animals examined in winter.

The lower limit of temperature that the winter animals could withstand was not determined. The upper limit of temperature was very distinct and sharp, there being a difference of only a few degrees between the temperature at which the animals appeared healthy and that at which they died. The individual differences at the thermal death point were slight, the larger animals appearing to be better able to withstand the sudden change to high temperatures than the small ones. There appeared to be no sex difference in the ability to withstand higher temperatures.

The experiments have shown, then, that there was a difference in response to temperature of the Emerita talpoida in summer and winter. At low temperatures, the winter animals had a higher oxygen consumption than the summer animals. In the middle temperatures, the oxygen consumption of the summer and winter animals was of somewhat the same order. At the high temperatures, there was a 10° difference in the thermal death point; the winter animals showed a sharp decrease in oxygen consumption beginning at 23° and ending with death at 27 to 30°, whereas the summer animals began to show a decrease in oxygen consumption at around 30° ending in death at 37 to 40°.

In winter the metabolic rate appeared accelerated above the summer rate at low temperatures. If the summer metabolic rate prevailed in winter, metabolism would be cut down to a very low level. With the winter type of metabolism, the depression would not be so severe. That the increased metabolism in winter is a significant adjustment of the animals to the season is indicated by the continued activity and growth of Emerita during the winter.

SUMMARY AND CONCLUSIONS

1. The life span of the Woods Hole Emerita talpoida is a litttle over 2 years. Growth is steady throughout the year.

2. Changes in temperature of 10° or less from the normal brought about rapid changes in oxygen consumption, but the oxygen consumption rapidly returned to normal when the animals were restored to the original temperature.

3. The oxygen consumption of both the winter and summer Emerita talpoida increased with increases in temperature, until heat injury set in, and then decreased. The maximum oxygen consumption of the animals in winter was at 23°; that of the animals in summer was slightly higher. The thermal death point of the animals in summer was approximately 10° higher than that of the winter animals.

4. The oxygen consumption of the smaller animals per unit of weight was larger than that of the larger animals, and the increase in oxygen consumption caused by temperature change was greater in the smaller animals.

5. The oxygen consumption in winter was greater than in summer at all temperatures below 20°C. After allowing for differences in size, the winter metabolism at 3° was about four times as great as the summer rate at that temperature.

6. It appears that Emerita talpoida Say from the Woods Hole area becomes adjusted to seasonal changes in temperature, so that the metabolism at low temperature in winter is kept up toward a level comparable to that of the warm summer months. The high rate of winter metabolism coincides with the continuation of growth and activity.

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THE INFLUENCE OF SEASON AND TEMPERATURE UPON THE OXYGEN CONSUMPTION OF THE BEACH FLEA, TALORCHESTIA MEGALOPTHALMA [1]

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ONE FIGURE

INTRODUCTION

Since the rate of metabolism of cold blooded animals varies with temperature, it is to be expected that their rate of oxygen consumption will show seasonal variations. Cronheim ('11) has indicated that the metabolism of the carp is greater in summer than in winter, and the same effect of season has been shown to occur in the killifish, Fundulus parvipinnis (Wells, '35), and in Mytilus edulis (Bruce, '26).

The relation of temperature to oxygen consumption appears to establish a factor which will dominate the seasonal activity of cold blooded animals. Two species from Woods Hole, in which this relation has been determined, have shown interesting differences. The relation of oxygen consumption to temperature in the cunner, Tautogolabrus adspersus, is the same in winter and summer, and the cunners leave their summer position and probably remain quiet in winter (Haugaard and Irving, '43). Emerita talpoida, on the other hand, has a more rapid metabolism at low temperatures in winter than in summer, so that the winter metabolism at 3° equals that in summer at 15°. The winter animals are active and grow, although at the summer rate of metabolism at low temperatures their activity would probably be slight (Edwards and Irving, '43).

On the same sandy beaches with Emerita talpoida at Woods Hole, but above the water line, one commonly finds the small sand flea, Talorchestia megalopthalma. It was thought to be of interest to determine if this air breathing neighbor of Emerita also made a significant metabolic adjustment to cold weather. The results of the investigation showed that Talorchestia megalopthalma went into a state of apparent hibernation beneath the sand in winter months, and that during this

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time the curve relating oxygen consumption to temperature was practically unaltered from its summer position. As a result of the depressing effect of temperature, the metabolic rate at winter temperature was lowered below a measurable level.

TECHNIQUE

The oxygen consumption of the animals at rest was measured by means of a volumetric micro-respirometer for air breathing animals (Scholander, '42). It was found by preliminary experiments that the Talorchestia used on the average 1 cu. mm. of oxygen per minute. The average experimental run lasted from 4 to 6 hours. Using a manometric apparatus, even with a gas volume as high as 20 cc., the oxygen tension would be lowered significantly in a few hours. The manometric apparatus is therefore not suitable for long continuous runs on animals having high oxygen consumption. The system used in the present investigation obviates the difficulty by having incorporated in it an oxygen supply which replaces continuously the oxygen used by the animal. This permits the use of small gas volumes which increases the stability and sensitivity of the apparatus as it renders the measured volume changes to be a larger proportion of the total gas in the system. The animal chamber used was 3 cc. in volume and the volume of the oxygen reserve bulb about $\frac{3}{4}$ cc. The animals were placed in a small, moist bag of plankton net attached to the oxygen port of the respirometer. Ascarite was placed in the bottom of the vessel to absorb carbon dioxide. The oxygen consumption was read directly from the micrometer scale. Without the animal the respirometer showed no oxygen consumption, so that the readings on the micrometer represented the true oxygen consumption of the animals.

At the start of each experiment, the machines were allowed 15 minutes to reach temperature equilibrium, and readings were taken every 10 minutes thereafter. The respirometers were kept in glass aquaria which were placed in a large water bath for constancy of temperature. At the end of the experiments the animals were wiped off with absorbent toweling and weighed. All figures were reduced to cubic millimeters of dry oxygen per gram of body weight per minute at 760 mm. of mercury and 0°C.

The water content of the animals was determined by weighing them and then placing them in a drying oven at 110°C. for 2 days and then weighing again. The remains were then digested with trypsin and weighed a final time to determine the proportion of digestible material.

ANIMALS

The Talorchestia used in these experiments were collected from Nobska Beach at Woods Hole, Massachusetts. Collection was made in July and August by catching the animals by hand as they hopped over the sand, or by digging a few inches beneath the surface to secure the larger animals. Considerable numbers were used for the measurements of size and metabolism shown in the tables, and the regularity of the data indicates that the samples are truly representative of the Talorchestia population of Nobska Beach. The summer animals were kept for short periods in the laboratory at room temperature, which was very much the same as that outside.

In the months of February and March, the animals were usually found curled up in little burrows beneath 4 feet of sand about 30 feet above the high tide mark, in a stratum of moist sand, decaying seaweed, and sticks. When disturbed in their natural habitat, they responded very sluggishly, but when warmed in the laboratory, they soon hopped about as actively as in summer. The animals used for winter experiments were kept at 3°C. At this temperature, they were inactive and curled up in burrows beneath the sand in which they were kept.

RESULTS

Digestible material

The analyses of water and digestible material were made in order to have a basis for comparisons of the oxygen consumption of Talorchestia megalopthalma with that of other organisms.

About forty animals were weighed and then dried in an oven at 110° for 2 days, or until constant weight was obtained. All the dried animals were then weighed and placed in trypsin at 37° for several days. The trypsin solution was changed several times. After digestion, the animals were washed, dried, and weighed. In the course of protein digestion, probably most of the non-chitinous substance was removed. Table 1 gives the results of the analyses of ten different animals picked at random. It can be seen that the proportion of water and digestible material does not change with changes in size, so that any of these dimensions used in referring to metabolism would give similar curves.

Oxygen consumption

The oxygen consumption of Talorchestia megalopthalma at different temperatures in winter and summer is shown graphically in figure 1.

Each point shows the average of steady rates for 10 minute intervals continued over 4 hours. The oxygen consumption showed a rise with increasing temperatures up to 32–33°C., and then a rather sharp decline, followed by the death of the animals at approximately 43°. That the thermal death point was so high in summer was not at all surprising, for many times at high noon in July, or August, when the sands were

TABLE 1

Water and digestible material content of Talorchestia megalopthalma.

WET WEIGHT IN GRAMS	DRY WEIGHT IN GRAMS	PER CENT WATER	WEIGHT OF DIGESTIBLE MATERIAL IN GRAMS	PER CENT DIGESTIBLE MATERIAL
0.135	0.037	72.5	0.024	17.7
0.143	0.052	63.6	0.033	23.0
0.176	0.057	67.6	0.036	20.4
0.193	0.063	67.3	0.038	19.6
0.201	0.059	70.6	0.039	19.4
0.269	0.077	71.3	0.049	18.2
0.285	0.087	69.4	0.056	19.6
0.385	0.121	68.5	0.079	20.5
0.433	0.117	72.9	0.080	18.4
0.341	0.094	72.4	0.067	19.6
		Mean 69.3% water		Mean 19.6% digestible material

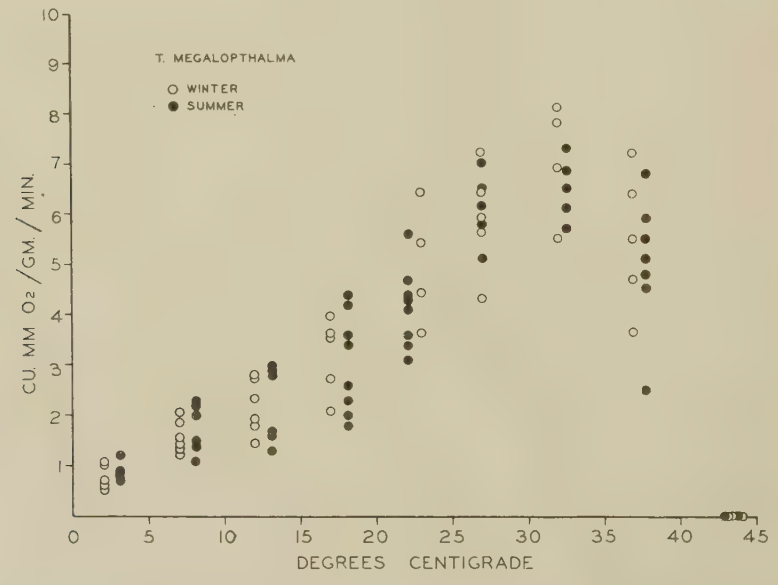

Fig. 1 The oxygen consumption of Talorchestia megalopthalama during winter and summer at various different temperatures. Each point represents the steady level of oxygen consumption of one animal over a period of 4 hours.

so hot that they burned one's feet, the sand fleas were seen actively hopping about on the sand surface. That the animals in winter still showed this same ability to withstand high temperatures was, however, of considerable interest.

It was noted in Emerita talpoida (Edwards and Irving, '43) that below 15° the winter animals showed a higher oxygen consumption than did the summer animals at the same temperatures. There was no sign of altered winter metabolism in Talorchestia megalophthalma. The data shows that at any given temperature the oxygen consumption of the animals in winter and in summer was practically identical. In both seasons when the temperature was below 10–12° the animals became

TABLE 2

Relation between size and oxygen consumption in Talorchestia megalopthalma.

WINTER			SUMMER	
Weight in grams	Oxygen consumed mm.³/gm./min.	Temperature in degrees C.	Weight in grams	Oxygen consumed mm.³/gm./min.
0.140	2.8	12–13	0.115	3.0
0.156	2.7	12–13	0.130	2.8
0.163	2.3	12–13	0.145	2.9
0.278	1.9	12–13	0.180	1.7
0.291	1.6	12–13	0.190	1.6
0.388	1.4	12–13	0.215	1.3
0.166	6.4	22–23	0.110	5.6
0.185	5.4	22–23	0.120	4.7
0.202	4.3	22–23	0.155	4.3
0.274	3.6	22–23	0.190	4.4
		22–23	0.200	3.9
		22–23	0.225	3.7
		22–23	0.230	3.3

sluggish and eventually completely inactive. With increasing temperatures the animals became increasingly active, until at the highest temperature they became desiccated and died. Observations of activity were made on animals separate from those used for the oxygen consumption experiments. Those used in the respirometers were undisturbed and quiet, and hence the increase in oxygen consumption resulted from the effects of temperature on the resting metabolism, and was not the result of increased movement.

That the spread of points at any one temperature results largely from the influence of size upon metabolism can be seen from table 2, in which the variation in metabolic rate with size is shown. The figures given in that table are those shown by the points on figure 1 at 12–13°

and 22–23°. In general, the smaller animals showed the higher oxygen consumption per unit of weight, although the relation between oxygen consumption and size was not strictly uniform. On the basis of a few experiments performed for comparative purposes, there seemed to be no significant difference in the oxygen consumption of males and females of the same size.

DISCUSSION

It seems that with the depressing effect of cold upon metabolism, Talorchestia can not remain active. In this respect, it resembles the cunner at Woods Hole, which is likewise sluggish at winter temperatures and disappears from its summer position (Haugaard and Irving, '43). Emerita talpoida from Woods Hole shows a distinct metabolic reaction to the winter season and has an increased metabolic rate at low temperatures. Along with this alteration of metabolic rate, Emerita remains active and grows during the cold months (Edwards and Irving, '43). This small series of animals examined shows that, if no alteration of winter metabolism occurs to offset the depressing influence of cold, the metabolic rate in winter is too slow to maintain activity. Animals, like Talorchestia, that fail to adjust metabolism to cold seem to have no alternative but to remain quiet or hibernate in winter.

SUMMARY

The oxygen consumption of Talorchestia megalophthalma from the Woods Hole area has been measured at different temperatures during the coldest months of winter and the warmest months of summer.

The oxygen consumption of the smaller animals was, in general, higher per unit of body weight than that of the larger ones.

The oxygen consumption of the animals in winter was the same as that for the animals in summer at a given temperature.

The results indicate that there is no essential modification of the rate of metabolism with season. The depressant influence of cold reduces the metabolism to such a low level that inactivity and apparent hibernation result.

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Contribution No. 319

PHYSIOLOGICAL ASPECTS OF SPRING DIATOM FLOWERINGS

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ABSTRACT

Experiments are described on the growth of the marine diatom, *Nitschia closterium*, under varying conditions of light, temperature, and nutrients. It is typical for the Nitschia population to pass through a cycle consisting of an initial lag, a period of logarithmic growth, a period of decreasing growth rate with the population reaching a peak, and a subsequent decrease to a low population level. A discussion is presented of the relative importance of certain physical and chemical factors at different stages in the population cycle.

A parallel study was made of the natural phytoplankton association in the Georges Bank region during a spring diatom flowering. It was found that many of the species passed through a population cycle during the flowering period which resembled that observed in the Nitschia cultures in the laboratory. Similarities were noted not only in the form of the population curves but also in their relationships with respiration, photosynthetic activity, changes in the population when stored in the dark, and possibly in the sinking rate. A final discussion attempts to synthesize the results of the two studies into an explanation of certain aspects of spring flowerings on a physiological basis.

INTRODUCTION

During the course of several cruises to Georges Bank in the spring of 1941, determinations were made of the growth of natural plankton associations taken from the surface waters and kept in bottles for two to four days. The results so obtained were later supplemented by a series of experiments on the growth of the diatom, *Nitschia closterium*, in the laboratory. In order to provide a general outline of diatom physiology, thus setting the stage for a discussion of factors involved in spring diatom flowerings, the Nitschia experiments will be presented first, together with a historical sketch of some of the previous contributions to the subject.

Certain general features of the growth cycle of diatoms were described in a qualitative way by Allen and Nelson (1910), a few extracts of which are given here:

"The medium [a modification of Miquel's formula] was found to give constantly satisfactory results. On inoculation from a persistent culture of such diatoms as *Thalassiosira*, *Skeletonema*, *Chaetoceros*, etc., a growth visible to the eye is obtained in about ten days, and then multiplication takes place very rapidly. In from three weeks to a month's time a very considerable growth will be seen making a brown flocculent mass at the bottom and back of the vessel containing the culture.

"In from three to four months the culture begins to show signs of

exhaustion and the frustules lose colour, but they do not, as in the case of sterilized outside and tank water, completely die off. A great number certainly do die, but some remain in a resting condition, and often, after a period of six months or so, these begin to multiply again and the culture regains its former vigour. This is probably due to the food stuffs contained in the dead frustules going into solution again, possibly by means of bacterial action. This periodicity in cultures is interesting in that it resembles what takes place in the ocean. Cultures in this medium will persist indefinitely, as far as our experience goes. The oldest culture in our possession is one of *Skeletonema costatum,* made at the very commencement of this work, dated April, 1905. Although the frustules in this culture are quite unrecognizable as any diatom now, on making a subculture in fresh Miquel a normal and healthy growth can always be obtained."

The only really quantitative determination by these authors is a series of counts of a young and vigorous culture of Thalassiosira growing in a hanging drop preparation, in which they showed that the growth curve approximated geometrical progression. But as will be seen later, more complete quantitative studies confirm the statements about the form of the growth cycle made by these authors.

More recent papers on diatom physiology have dealt chiefly with nutrient requirements and the effect of light on growth. Among the former may be mentioned Harvey (1933, 1937, 1940), Ketchum (1939, 1939a), as well as Von Brand and Rakestraw (1940, and a series of earlier papers) who dealt primarily with the nitrogen cycle in sea water but also showed something of the nitrogen requirements of Nitschia. A number of workers, including Marshall and Orr, Gran, Braarud, and Jenkin have used diatom cultures to study various aspects of photosynthesis in the sea, and more exact laboratory experiments have been done by Stanbury (1931) on the effect of light on growth rate and by Barker (1935) on photosynthesis and respiration. They have added much to our knowledge of diatom physiology, particularly of the earlier phases of growth. The complete life cycle—the form of the growth curve, its relations with photosynthesis and respiration, and the effect of certain environmental factors at different stages of growth—have yet to be described in detail. The present experiments are intended to fill some of the widest gaps in this subject, in such a way that the results may be of use in interpreting data obtained from the natural environment.

EXPERIMENTS ON *NITSCHIA CLOSTERIUM*

General Characteristics of the Material

The material used in the experiments was a variety of *Nitschia closterium* obtained from Long Island Sound. During the course of some experiments on oyster feeding, Dr. V. L. Loosanoff of the Milford Biological Laboratory (U. S. Fish and Wildlife Service) enriched a large tank of Sound water with commercial fertilizer. An abundant growth of Nitschia developed, together with a few other diatoms and several species of flagellates. From some of this material a persistent culture was prepared, containing, beside the dominant species, bacteria and a few minute flagellates.

Measurement of fifty cells of a Nitschia culture yielded the following results: total length 30.7 to 38.4 μ, with a mean of 33.90; length of the central expanded portion of the cell containing the chromatophores 10.2 to 16.4 μ, mean 13.55; maxium width 2.6 to 4.1 μ. The size of the cells in the stock culture did not vary significantly in a period of five months during which it was kept in a state of continouous active growth; nor did it vary during the course of an experiment in which the cells were measured every few days from near the beginning to the peak of the growth curve. However, the Nitschia growing in Long Island Sound at the time when the original culture was started were considerably larger, with a total length of about 105 μ, a cell body length of 22 μ, and a breadth of 4.1 μ. It is not known exactly how long it took them to decrease to the constant size noted above, but the period was not more than a month and a half. This initial decrease and the attainment of constant size are in accordance with results obtained by Allen and Nelson (1910, 461) with several species of diatoms.

In a young, vigorous culture most of the cells move freely. They are straight and symmetrical. The chloroplasts are of a uniform green color and nearly fill the central body of the cell, and the clear spaces between individual chromatophores are narrow, except during and for a short time after division, when the daughter cells show a distinct, clear, transverse band in the middle of the cell, due, supposedly, to the fact that the chromatophores do not immediately regain full size after division.

In every culture there are cells that have a somewhat debilitated appearance. The chromatophores are darker than normal and con-

tracted, and in extreme cases they appear only as small dark spots within an otherwise colorless cell. The cell wall may be distorted, the most common abnormality being a distinct curve in the end processes. Such cells move little if at all. They may go through a few cell divisions but frequently do not. Their usual fate is to fade gradually until the frustules show no visible evidence of activity or of the presence of organic substance.

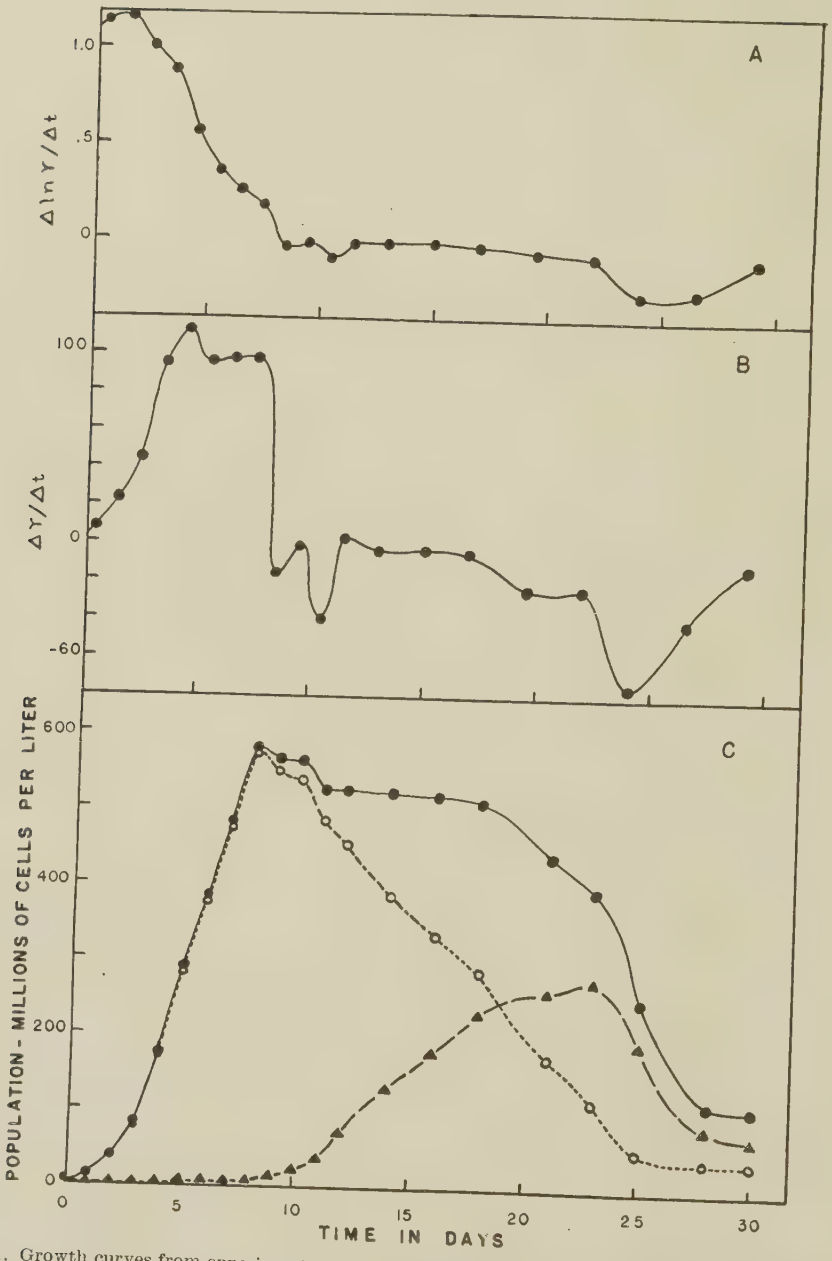
These so-called "senile" cells occur in relatively small numbers in actively growing populations. They appear in large quantity about the time the population reaches its peak and often are numerically dominant during the later period when the culture is dying away. Their numerical importance and their relation to the general form of the growth curve justify distinguishing them from the more active members of the population. However, it must be remembered that the use of the term "senile" is somewhat arbitrary. Morphologically, these cells represent various stages of a transition between complete normality and death. Presumably the same may be said of their physiology. As noted above, they occasionally are known to reproduce a little after the onset of senility, and at quite late stages some of them can be revived and transformed into active cells by transferring them to fresh culture medium.

The Growth Curve

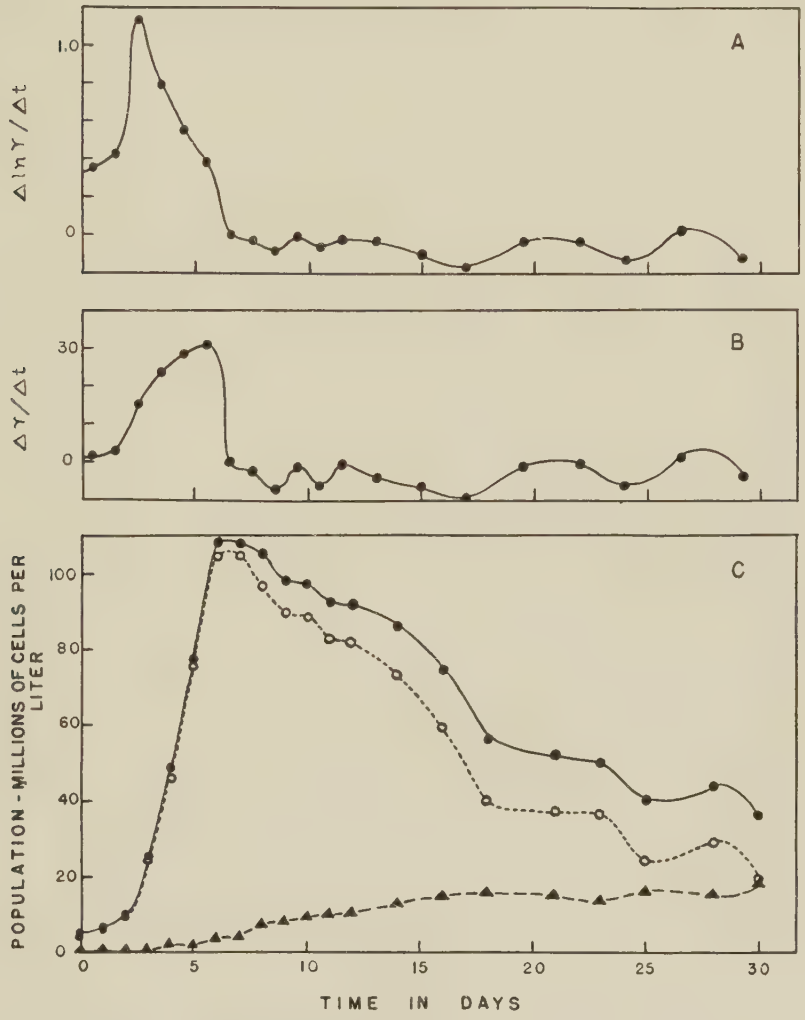
General Features

Culture medium for the first experiment was prepared by filtering Long Island Sound water, "pasteurizing" it by heating to 70° C., and enriching it with a few milligrams of a commercial fertilizer recommended by Dr. V. L. Loosanoff, who had conducted a large number of experiments on the use of various nutrients in promoting the growth of Nitschia and other micro-organisms. A liter of the medium was seeded with five million cells of an actively growing stock culture, placed in a sub-cellar which had a fairly uniform temperature (the temperature of the culture was measured every day and found to be 13.0 $\pm$ 1.1° C.), and illuminated by a one-hundred watt bulb at a distance of two feet, the rays from which first passed through a small, flat aquarium jar full of water, which absorbed most of the radiant heat.

The resulting growth curves are shown in Figure 1. Figure 1C is the population plotted against time. Figure 1B is the absolute rate



1. Growth curves from experiment 1. A is the relative rate of growth and B the absolute rate. C is the population curve, in which the total population is indicated by dots and solid line, active cells by circles and dotted line, and senile cells by traingles and broken line.



2. Growth curves from experiment 2. Arrangement and symbols as in Figure 1.

of growth of the total population expressed as $\frac{\Delta Y}{\Delta T}$, the daily increment in the number of cells in the population. 1A is the relative rate of growth $\frac{\Delta \ln Y}{\Delta T}$, the daily increment in the logarithm of the total population.

The beginning of the population curve had the usual sigmoid form, an initial increase in the form of geometrical progression, with an increasing absolute rate and a constant relative rate. Then, as is also usual, the relative rate began to drop off and the population curve passed the inflexion point, represented by the peak of the curve for the absolute rate of growth. At this point all resemblance to the sigmoid type of population curve ended. Instead of coming slowly to an asymptote, the rate of growth remained constant for a few days and then abruptly dropped to a negative value, indicating a sharp peak in the population level, followed by a gradual decrease.

Although this is not the usual form of growth curve, it is not unique, having been observed particularly in bacterial populations in which the peak coincides with the beginning of sporulation. But the mode of formation of the peak of the diatom growth curve has several unique features which are discussed later (pp. 19–26) and which are related to general marine economy.

Further events in Figure 1, which are fairly typical though not universal in the minor details, may be summarized as a steady decrease in active cells and a corresponding increase in senile ones, leading to a peak in the latter followed by a decrease as they begin to die, and finally a tendency to establish a population equilibrium at a low level.

A second experiment is illustrated in Figure 2. Here all the conditions were the same as in the preceding experiment except that no fertilizer was added. The poorer quantity of nutrients is reflected in a maximum population of slightly less than one-fifth that recorded in Figure 1; there were also certain modifications of the form of the growth curve. Most important of these is the fact of an initial lag, shown most clearly in Figure 2A, the relative growth rate. At its peak, between the third and fourth days, the rate of growth was of the same order of magnitude as the maximum rate observed in the first experiment, but it was achieved only after two days' growth at a slower rate.

Another difference in the two experiments is the fact that after the population had passed its peak, the senile cells, although seemingly

produced from the active cells as in experiment one, did not persist as long and consequently did not reach a peak, but rather established an asymptote. Both these phenomena, an initial lag in growth and the quick disappearance of senile cells, are fairly common.

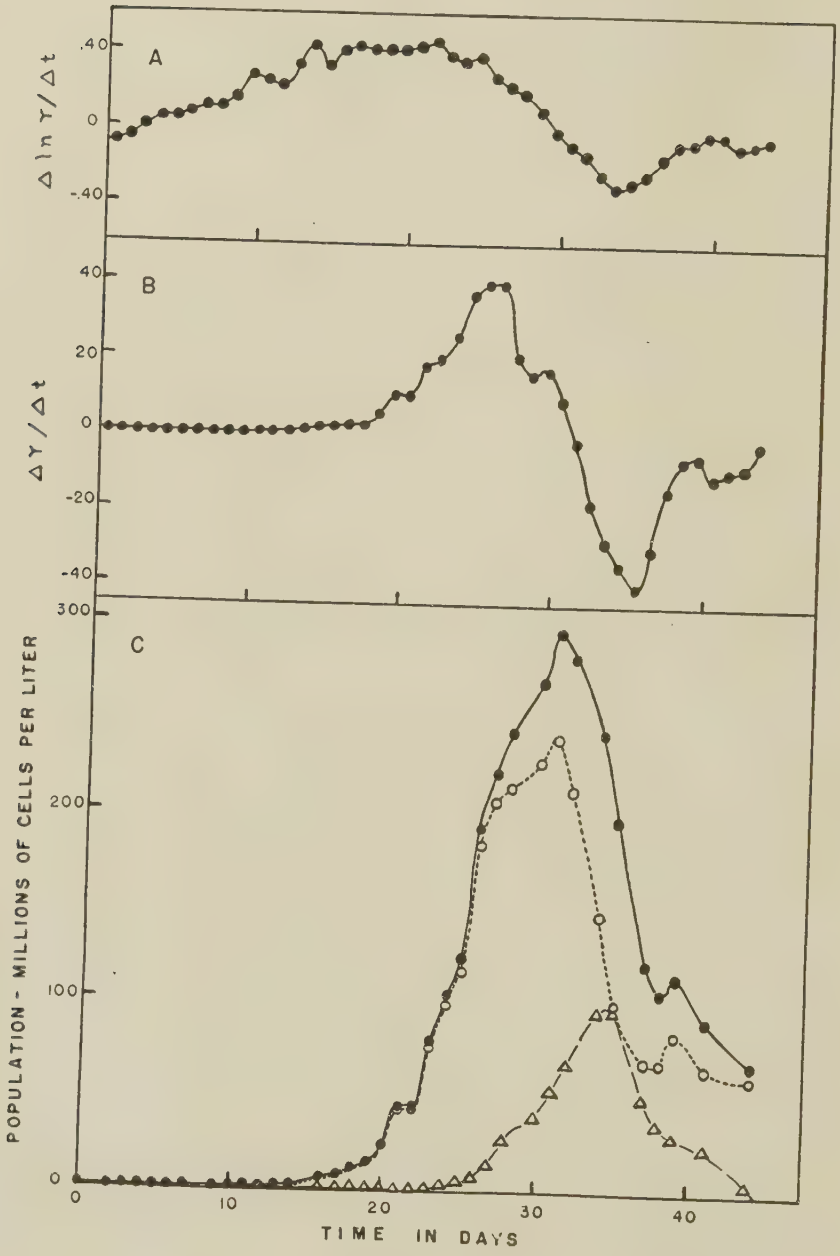
Photosynthesis and Respiration

Figure 3 shows the growth curves of a Nitschia culture in the same medium used in the first experiment, but seeded with a small concentration of cells (600,000 per liter) and grown at room temperature in the light from a north window. There was a long lag period lasting about fifteen days and starting from a negative level; *i. e.*, there was a decrease in the population during the first two days. The population curve was somewhat less regular than those generally obtained under constant conditions. The growth rates were highly irregular, and the curves presented for $\frac{\Delta Y}{\Delta T}$ and $\frac{\Delta \ln Y}{\Delta T}$ have been smoothed by fives. The essential features, however, were similar to those in the previous figures.

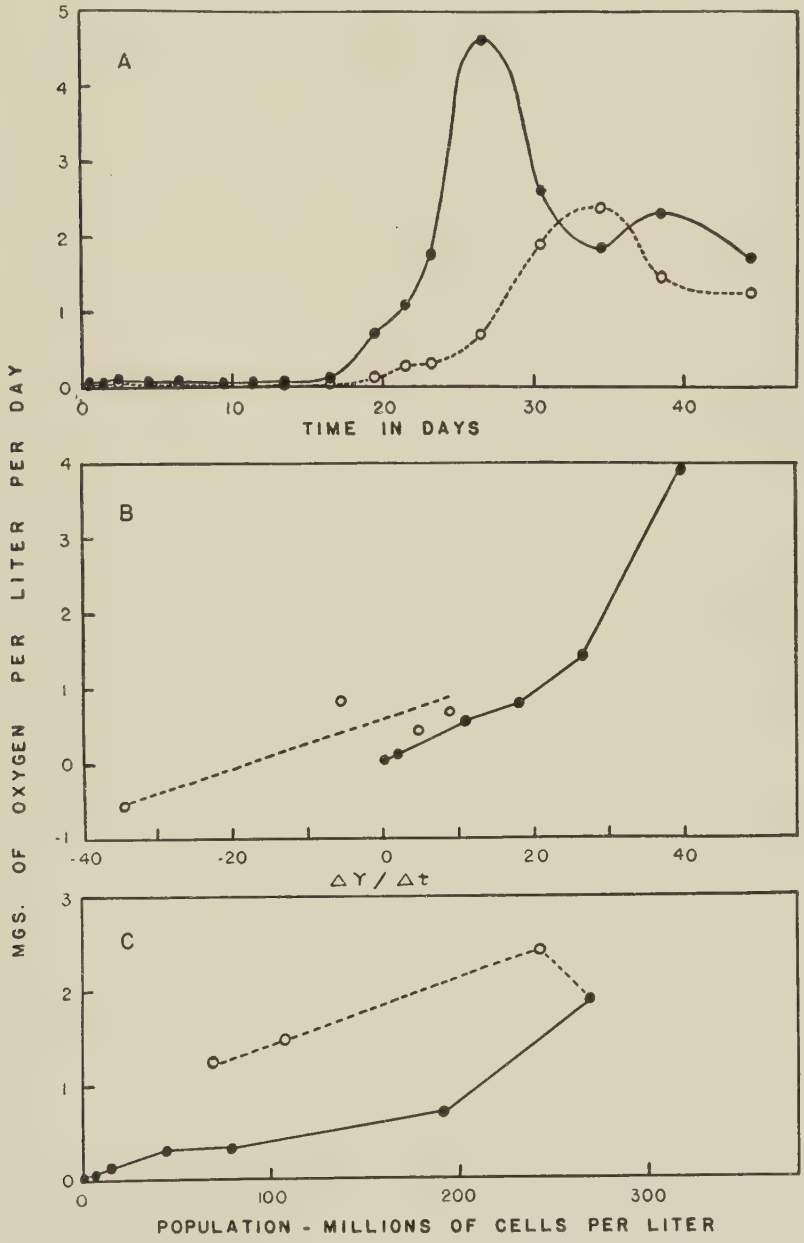
At intervals during the course of the experiment measurements were made of the photosynthesis and respiration of the culture. This was done by filling three oxygen bottles with the culture, measuring the oxygen content of one of them immediately, and analyzing the other two twenty-four hours later, after one had been exposed to the same conditions of light and temperature as the main body of the culture, and the other had been stored in the dark. From these measurements, records were obtained of respiration, increase of oxygen in the light bottle, and photosynthesis over one-day periods. These records are plotted in Figure 4A; dots connected by a solid line indicate photosynthesis, and circles and dotted line are respiration values.

Comparison with Figure 3 shows that there was some relation between photosynthesis and the population curve, but the maximum photosynthesis corresponded not with the peak of the population curve, but rather with the maximum rate of absolute growth. Nor was there any very good relation between total population and respiration. The latter reached its maximum somewhat later, at the time when the senile cells reached their peak.

These relationships are shown somewhat more clearly in the remainder of Figure 4. 4B is based on the theoretical assumption that the products of photosynthesis are used for two purposes: the energy



3. Growth curves from experiment 3. Arrangement and symbols as in Figure 1.



4. A. Photosynthesis (dots and solid line) and respiration (circles and dotted line) in experiment 3. B. Difference between photosynthesis and respiration plotted against absolute growth rate. Dots and solid line represent the culture before it reached its maximum growth rate; circles and dotted line, afterwards. C. Respiration plotted against population. Dots indicate events prior to maximum population; circles, the culture after its peak.

used for metabolic processes (which is equivalent to the respiration), and the carbon that goes into, and should be related with, the production of new cells. Accordingly, in 4B the difference between total photosynthesis and respiration is plotted against the absolute growth rate. Dots and solid lines indicate records before the achievement of the maximum photosynthetic and growth rates; circles and dotted line show subsequent developments. During the first part of the experiment the assumption was justified. There was a fairly good linear relationship in which an increase in oxygen of one milligram corresponded to a production of about 20 million cells. Later on, however, as the growth rate approached a maximum, about twice as much photosynthesis was required for the same amount of increase in the population. In senescence the data are not adequate to permit accurate description, but there seemed to be a further change in the relation, with a decrease in the slope of the curve.

In Figure 4C respiration is plotted against the total number of cells. Before the peak of the growth curve was reached there was an increase in respiration with population, but it was roughly sigmoid, with the slowest increase occurring at the time when the population was increasing most rapidly. During senescence the respiration decreased with the population but at a relatively higher level, as indicated by the circles and dotted line.

The respiration per unit population was greatest during the lag period, ranging from about 0.03 to 0.11 mg. per million cells per day. The wide range in values may be real but more probably is due to analytical errors involved in the determination of very small differences in oxygen content. The respiration rate began to drop at the same time the population began to increase, reaching a minimum point of 0.004 mg. per million cells per day when the absolute rate of growth was at its peak. Thereafter it increased, reaching a value of about 0.02 mg. during late senescence.

The relationship of photosynthesis and respiration to the population curve can be understood more clearly if an attempt is made to express them in comparable units, such as milligrams of carbon. This can be done easily in the case of photosynthesis and respiration. The measurement of the carbon content of the population is more difficult, especially when the latter is small. It is necessary to attempt the estimate on the basis of a few measurements of total organic matter made during the logarithmic growth period, when the population was

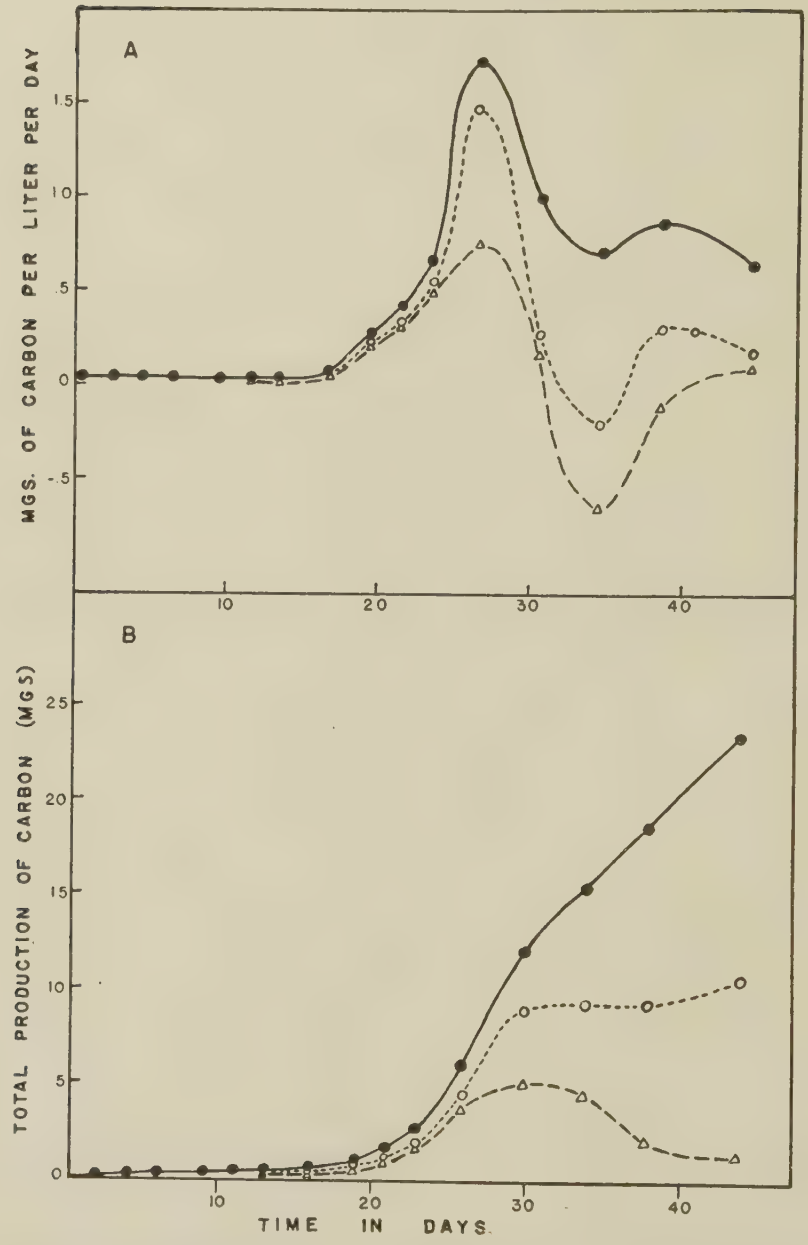
large. This does not necessarily represent the true organic matter content of the population at all stages of the growth cycle.

According to these measurements the dry weight of organic matter per million cells of a rapidly growing population was .038 mg., from which one may assume a carbon content of about .019 mg. The carbon equivalent of the oxygen figures used thus far in discussing photosynthesis and respiration is of course derived from the chemical equation and is .375 times the weight of oxygen.

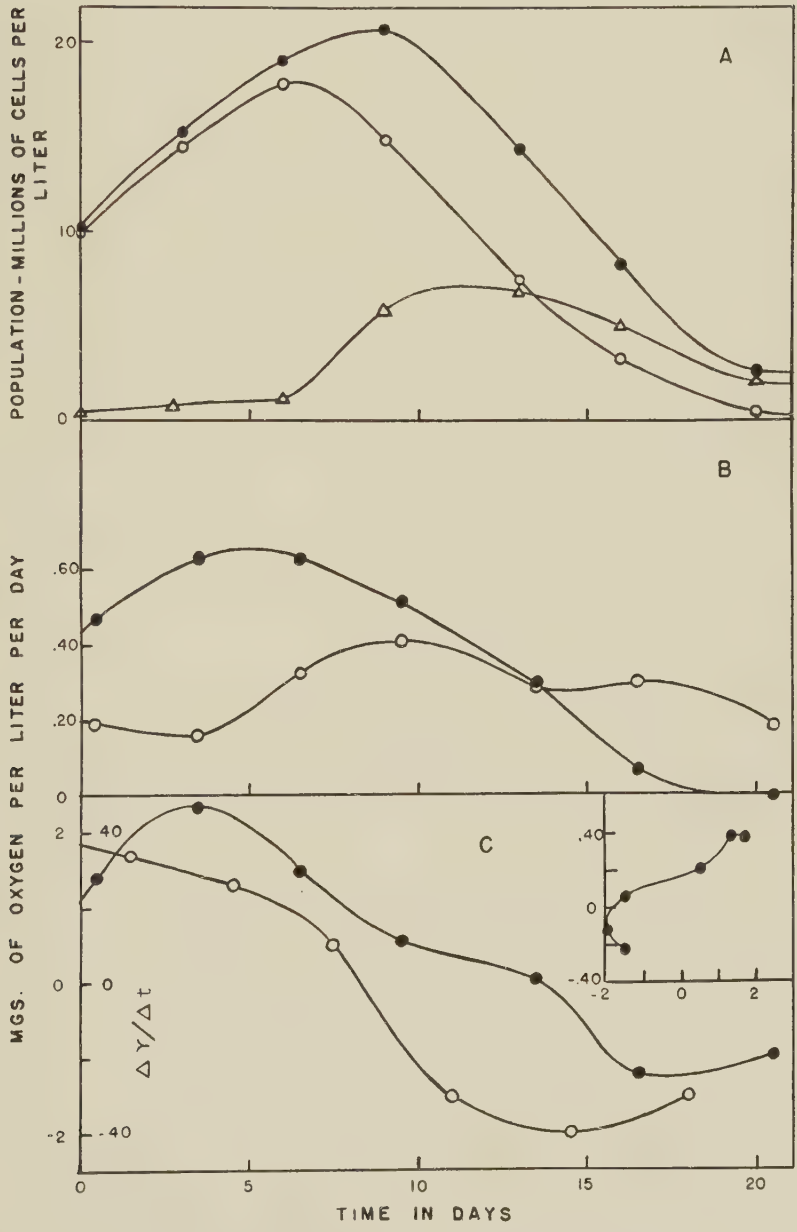
These values are used in Figure 5A to compare the photosynthetic rate (dots and solid line), the difference between the photosynthetic and respiratory rates (circles and dotted line), and the absolute growth rate of the population (triangles and broken line). The difference between the upper and middle curves is of course attributable to respiration, probably largely Nitschia respiration but perhaps containing a significant amount of bacterial or flagellate respiration, particularly after the peak, when the diatoms were dying and there was much organic matter available for scavengers. During the early part of the growth cycle there was little difference between the productivity as expressed by the difference between photosynthesis and respiration and the observed productivity of the population. Later, however, there were marked discrepancies which on theoretical grounds can be ascribed to two factors, the relative quantitative significance of which unfortunately cannot be evaluated. One of these factors is the accumulation of carbon in the cells, a situation that is quite possible when scarcity of nutrient salts prevents the products of photosynthesis from being utilized in the production of new cells. The second factor is the lag period between the death of the cells and the decomposition of their organic matter. If the latter were decomposed immediately, the result would of course be an increased respiratory rate and a corresponding decrease in the difference between photosynthesis and respiration.

The total production of the culture is obtained by integrating the rate curves. These are shown in Figure 5B. They show that throughout the entire growth cycle the total amount of energy that had been fixed continued to increase, although in the later stages the increase was balanced by respiration so that the accumulated energy content reached a fixed level, and furthermore the population decreased through death, leaving much of its carbon content as detritus.

A second experiment on photosynthetic and respiratory rates is



5. A. Carbon production rate calculated from experiment 3. Dots, photosynthesis; circles, difference between photosynthesis and respiration; triangles, population. B. Total carbon production (curves in A integrated). Symbols as in A.



6. A. Population curve in experiment 4. B. Photosynthesis and respiration. Symbols in A and B as in figures 3 and 4. C. Difference between photosynthesis and respiration (dots) and absolute growth rate (circles). These are plotted against each other in inset.

shown in Figure 6. This experiment was rather abortive in that the medium was unfertilized water taken from Long Island Sound in September when the nutrient content was low and permitted little growth. The results, however, are comparable to those shown in the preceding figures. 6A is the population curve. In 6B the curves for photosynthesis (dots) and respiration (circles) show approximately the same relations to the growth curve as in Figure 4. In 6C the difference between photosynthesis and respiration (dots) is in fair agreement with the absolute growth rate (circles). The small inset shows these plotted against each other. The only major differences are that the rate of respiration per unit population and the photosynthetic activity per unit increase in population were both relatively high in the second experiment.

Similar changes in photosynthetic activity with the growth of the culture have been noted by Barker (1935), who reported that the photosynthetic rate during the period of active growth was about five times as high as it was twenty days later, and that it decreased with the decrease in motility of the culture, also that there was no correlation with chlorophyll concentration.

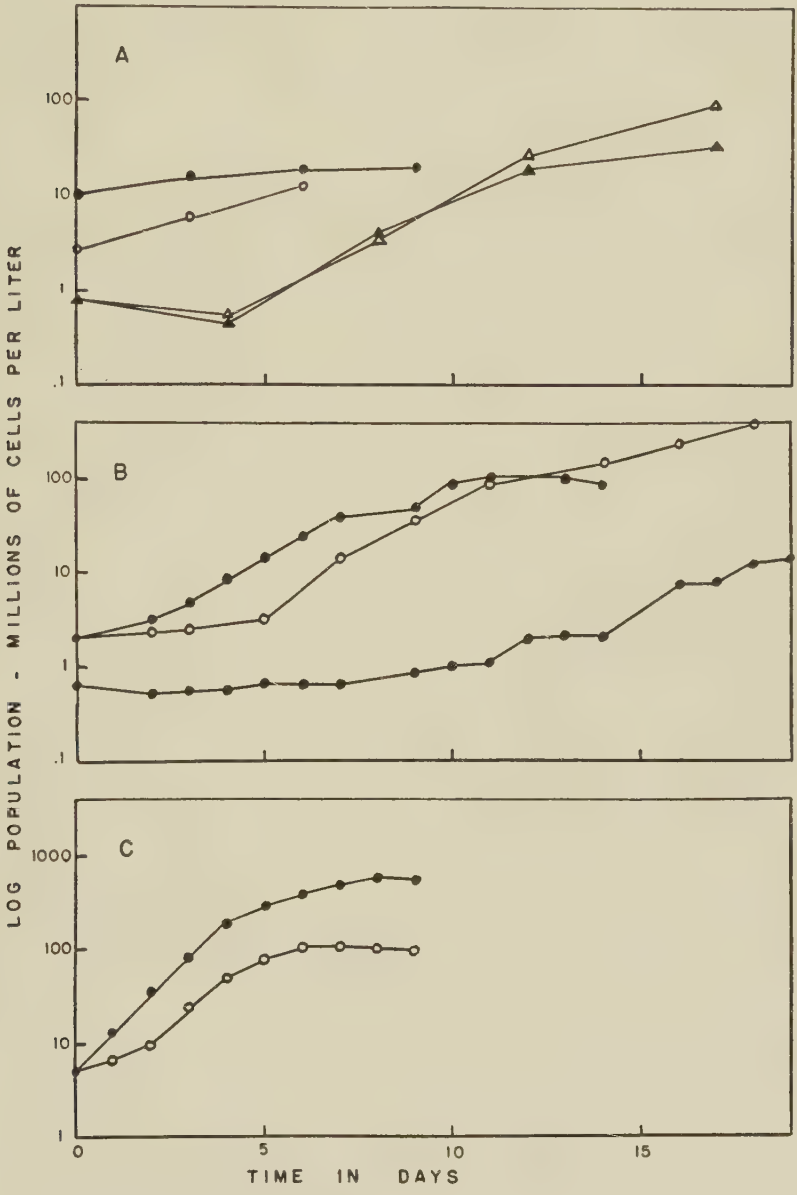
The Initial Lag

There is no intention, at present, to attempt an exhaustive study of the factors involved in the peculiar diatom growth cycle. It seems desirable, however, to bring together such facts as are readily available on the subject. In so doing attention is centered upon the successive phases of the cycle—the initial lag, the logarithmic curve, the peak, and senescence.

In the four experiments described thus far, two of them, namely those illustrated in Figures 2 and 3, showed an initial lag; the other two did not. It therefore appears to be a common though not universal phenomenon.

The situation is somewhat clarified by Figure 7, which shows logarithmic plots of the beginning of a number of experiments, those which have already been described and others which will follow later.

In 7A, the curve drawn with solid dots is taken from the experiment previously shown in Figure 6. It showed no evidence of a lag period, nor did the one just below, illustrated by circles, which was also an example of growth in nutrient-deficient, early autumn water. The next two experiments had a somewhat more adequate nutrient content



7. Logarithmic plots of initial growth curves. For further description see text.

as indicated by the larger total growth. The one with solid triangles was natural water taken early in October; the one with open triangles was the same, enriched with nitrate and phosphate. Both showed a decided lag period. In 7A as a whole, there is no evidence that the adequacy of the medium (as evidenced by later growth) had any power to alleviate the initial lag.

The three experiments in 7B were all grown in the same kind of medium, sea water with artificial fertilizer added. The initial lag ranged from very slight to extremely pronounced. In all these experiments physical conditions were approximately the same. The one apparent difference was the initial concentration of cells, which appeared to be the most important factor in determining the presence or absence of an initial lag, though certainly not the only one. There was some individual variation that cannot be explained easily, for the two experiments in 7B that started with the same concentration of cells, the same medium, and at the same time, varied considerably in the length of their lag periods. Also 7C, which is the logarithmic plot of the experiments previously illustrated in Figures 1 and 2, shows that nutrients may make some difference in experiments that are otherwise identical.

Without inquiring into the cause of the lag, which leads into such complex and controversial subjects as "autocatalysis" and "conditioning," upon which the present discussion can bring to bear no really critical evidence, it is still possible to introduce a few pertinent facts about the processes involved.

It has already been noted that the respiration per unit population was greatest during senescence and during the lag period, and least at the time when the population was growing most rapidly. Another point of interest in this connection is the fact that the senile cells constituted about 10 to 20% of the total population during the lag period, less than 10% during the phase of logarithmic growth, and increased again in senescence. In these two respects the initial lag resembled senescence. The photosynthetic rate, however, was relatively higher in the lag than in the late stages of the growth cycle, averaging nearly twice as much as the respiration and sufficient nearly to double the population every day. It would seem, therefore, that the lag can be characterized as a period of rapid growth and rapid death, with the former gradually increasing and the latter decreasing, so that the rate of increase in the population mounts steadily.

Logarithmic Growth

The logarithmic growth period, the linear sections shown in the logarithmic population curves in Figure 7, is perhaps best defined as that part of the growth curve in which the relative rate $\frac{\Delta \ln Y}{\Delta T}$ is constant. This was shown particularly well in Figure 3A, in which there was a gradual increase (the lag period) during the first thirteen days, a relatively constant level (logarithmic growth) from the thirteenth to the twenty-second day, and a subsequent decrease.

In most of the experiments conducted at room temperature with natural light, $\frac{\Delta \ln Y}{\Delta T}$ became constant at about .40 to .60. In two experiments in which the supply of nutrients was very poor, the maximum rate attained was somewhat lower, but in general the effect of the quality of the medium was not to alter the maximum rate of growth, but rather to change the length of time that it persisted.

Altering the physical conditions of the experiment appeared to change the rate of growth. The experiment figured in 1A was performed with the same medium as 3A. With continuous illumination and a lower temperature, the rate was nearly three times as high (1.0–1.2). With a poorer medium but with the same conditions of light and temperature, the same high rate was attained, but it persisted only one day.

Generalizing on these results, it may be said that with an adequate supply of nutrients, the Nitschia cultures pass through a period of logarithmic growth. The growth rate at this time is apparently the maximum attainable under the particular physical conditions of the experiment—the amount of photosynthesis that can be carried on with the light available, and the amount of the energy produced that is drained away by respiratory requirements at that particular temperature. Increasing inadequacy of nutrients sooner or later reduces the growth rate, but Nitschia growing in a relatively poor medium is likely, for a short time early in the period, to achieve the maximum rate possible under the prescribed physical conditions.

Decreasing Growth Rate and Peak of the Population Curve

The foregoing paragraphs have implied that exhaustion of nutrients leads to a decrease in the growth rate. While this is quite true, it will be evident that any adverse factor or set of factors, physical or chemi-

cal, can limit growth and bring on the beginning of senescence. The discussion therefore begins with a description of the mechanics of chemical limitation, proceeds to an analysis of the effects of physical factors, and ends with a general discussion of limiting factors.

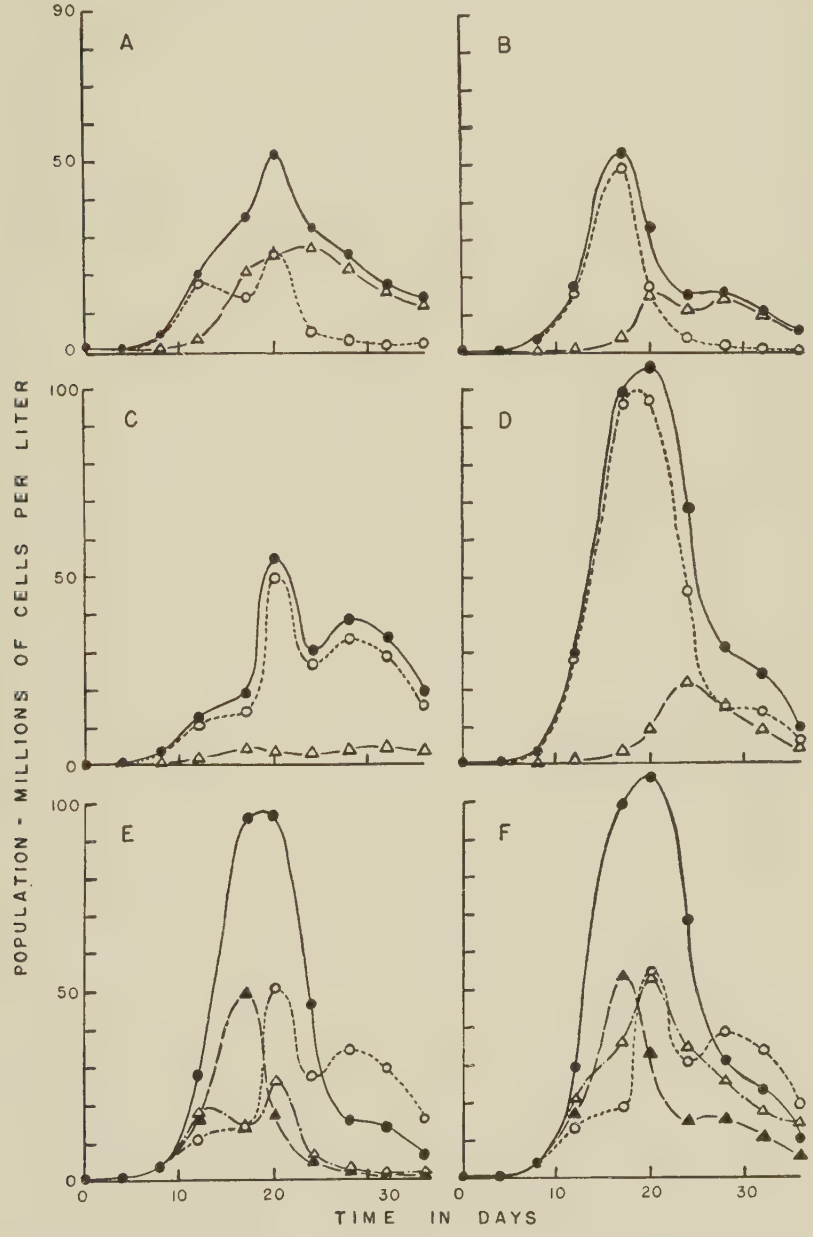
a. Chemical limitation

Two experiments suffice to introduce the subject of chemical limitation. The first is the growth of Nitschia in a poor culture medium (Long Island Sound water collected in September) with or without the addition of small quantities of nutrient salts. The initial concentration of cells was one million per liter. The kinds and quantities of salts added were as follows: phosphate, 20 mg.-atoms of P per m^3; nitrate, 300 mg.-atoms of N, ferric chloride, 5 mg.-atoms of Fe; manganese chloride, 20 mg.-atoms of Mn; and gallium chloride, 4 mg.-atoms of Ga per m^3. The combinations of salts used and the resulting maximum populations are shown in Table I. The two sets of figures indicate duplicate experiments.

Table I. Growth of Nitschia in Sea Water with Nutrient Salts Added.

Material added	*Maximum population (millions of cells per liter)*	
Control	8	9
P	8	14
N	20	8
P + N	29	35
Fe	7	3
Fe + P + N	38	40
Mn	13	14
Mn + P + N	33	35
Ga	21	24
Ga + P + N	33	28
Fe + Mn + Ga + P + N	85	79

It is evident from the table that in the majority of cases addition of one substance had a stimulating effect on growth. This was true of half the N and P experiments and all of those with Mn and Ga. With iron added there appeared to be a reduction in growth, but this may have been a special case due to precipitation or reduction of the ionization of the phosphate. In general, the more substances added the better was the growth, although there were two exceptions, namely that Ga or Mn in addition to N and P did not produce a significantly larger population than N and P by themselves. It is evident from this



8. Growth of Nitschia in various culture media. A. Control. B. Phosphate added. C. Nitrate added. D. Phosphate and nitrate added. Symbols as in Figure 1. E. Active, and F. total cells from the four experiments plotted together.

experiment that Nitschia does not follow very closely the classic Law of the Minimum. On the contrary, some stimulation of growth may be obtained with the addition of any one of a number of necessary substances.

Another experiment on the addition of N and P is shown in Figure 8. 8A is the control; dots and solid line indicate total population, circles and dotted line the active cells, and triangles and broken line the senile cells. 8B is the same water with 20 mg.-atoms of P per m^3 added; 8C contains 300 mg.-atoms of nitrate N; and 8D contains both P and N. 8E is a comparison of the number of active cells in the four experiments, and 8F is the same for the total cells.

The figure shows two unusual variations in growth curves. For the queer form of 8C, the nitrate curve, there is no adequate explanation. It is probably a real abnormality, but this was one of the first experiments, and early imperfections in technique may have led to erroneous counts. The other abnormality, the small and irregular curve for active cells and the relatively large number of senile ones in the control, is probably real, for it has been observed in other cases in which the cells were grown in deficient media. It was the first evidence, later confirmed in several ways, that the so-called senile cells are not always dying ones, but may be only somewhat deficient, and can on occasion divide a few more times before they die.

In this experiment addition of N or P did not materially increase the total population, although each of them approximately doubled the number of normal, active cells. Addition of both N and P caused pronounced improvement in growth.

The mechanics of this vague and rather elastic relationship between Nitschia and its essential elements can be explained quite adequately by the work of Ketchum (1939), who found that Nitschia could continue growing after the phosphorus in the medium was exhausted, with each division reducing the phosphorus content per cell until it might become as little as 20% of the normal content. The experiment in Table I of Ketchum's paper is of interest in this respect. It shows that the population approximately doubled after the phosphate was exhausted, but with a greatly reduced relative growth rate. It would appear from this set of data that the period of logarithmic growth ends at or near the time when one or more essential nutrients are exhausted, after which the population continues to grow more and more slowly as it becomes gradually more deficient in essential food

accessories until finally the death rate equals the division rate, and the peak has been reached.

b. Physical limitation

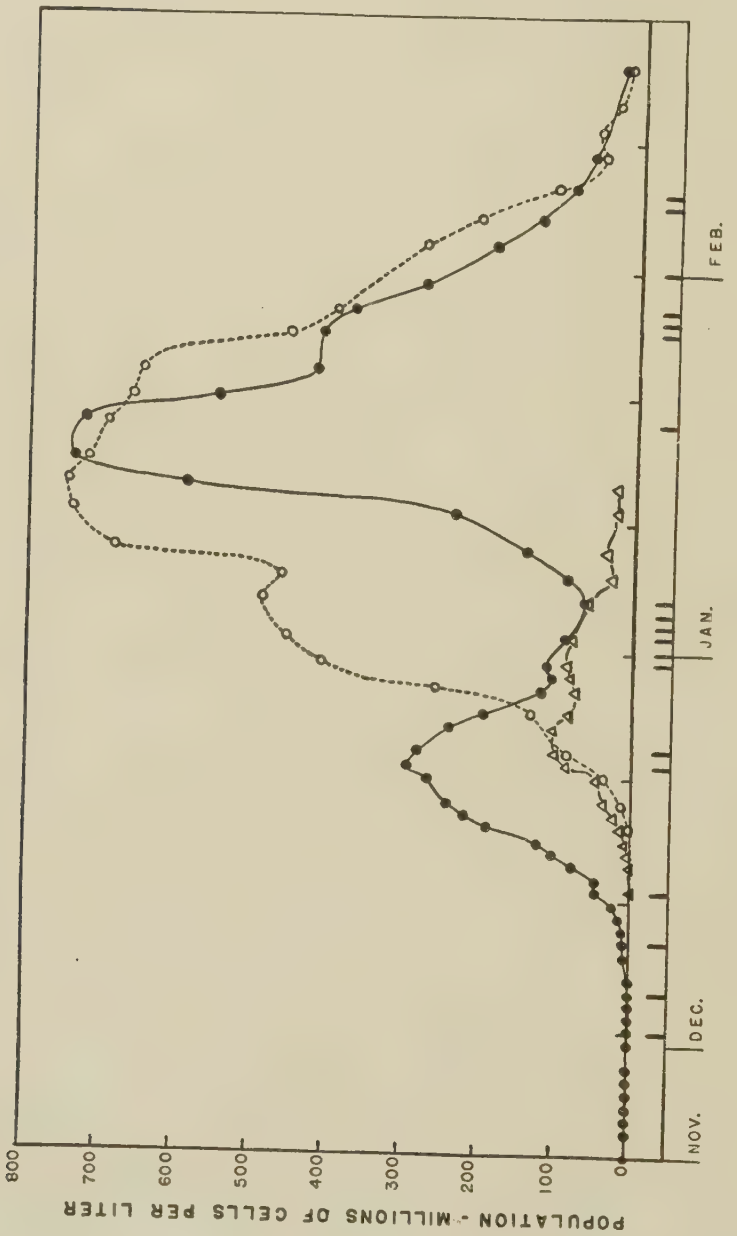
The effect of light on Nitschia growth frequently can be detected in irregularities that occur when the cultures are grown in natural illumination. This is shown in Figure 9. The triangles represent an experiment with natural water; the other two were enriched with commercial fertilizer. The short lines at the bottom of the graph indicate days when the sky was overcast, with rain or snow.

In the early stages of the first experiment (dots and solid line) there was some evidence of a decrease in the growth rate on dark days. In the rainy period of the thirty-first and thirty-second days, one experiment suffered a slight decrease in growth rate, and the other two went into senescence. The first recovered completely in the sunny days that followed, and the other two showed slight signs of renewed growth. More rain and snow from the thirty-ninth to the forty-third day put all the experiments into senescence, from which the two fertilized ones later recovered and were probably limited at last by the exhaustion of nutrients.

The first experiment (dots and solid line) was grown in a five-gallon carboy until the forty-fourth day (it was used for one of the experiments on photosynthesis and respiration), after which the remains were put in a liter flask. Probably the large amount of medium had an especially important effect in this experiment in cutting down the effective radiation reaching each cell. However, in spite of irregularities in the degree of senescence produced and the amount of time required for recovery, it is quite apparent that decreases in light intensity can reduce the rate of growth during early stages, and somewhat later in the cycle can change the population from a state of active growth to senescence long before it would otherwise occur.

Some might object to the term "physical limitation," which is used as the heading of the section. To be sure, physical factors are generally considered to condition rather than limit. That is, within broad ranges they determine whether an organism can grow or cannot grow, and within more narrow limits they determine the rate of growth but not the total amount of growth that can take place.

Practically speaking, however, physical factors can in certain cases simulate true limitation. A Nitschia culture growing under constant



9. Influence of cloudy days (vertical lines at bottom of graph) on growth of Nitschia cultures.

illumination becomes gradually thicker and more opaque. The amount of light reaching each cell steadily diminishes, and no matter how adequate the nutrients may be, the growth rate falls and the culture goes into senescence. The form of the curve is indistinguishable from the more customary curve due to nutrient limitation; in fact it is a biological exhaustion of light, just as the other kind is a biological exhaustion of nutrients.

c. The laws of limiting factors

It is evident from this and from practically every other recent physiological work on the subject that Liebig's Law of the Minimum and Blackman's Law of Limiting Factors are much too arbitrary to be applied with any degree of accuracy. To the first there is the objection that factors do not act independently; organisms are generally somewhat elastic in their requirements, and when most of the factors in their environment are favorable, growth may continue long past the point where one or a few factors become adverse. To the second, there is an indefiniteness that eludes practical application, for a chemical factor may begin to limit growth before it is completely exhausted (when growth depends on absorption which behaves according to the mass action principle), and on the other hand, as stated above, some growth may occur after exhaustion of the nutrient.

To a physiologist working with heavy cultures in strongly fertilized media, these objections might appear trifling, for they apply to a relatively narrow transition point in his experiments. To the ecologist, who deals almost exclusively with the borderline zone in which one or several factors limit growth, too much dependence on the classical laws can lead to sorry results. The ecologist cannot be sure that phosphate is the most important limiting factor even though his test for it is negative, nor can he be sure that it is not limiting when there is a little present. In such instances a practical experiment is more valuable than theory. For such purposes the well-known Schreiber method and its recent modifications are useful, and with care the same results sometimes can be obtained by statistical analysis of the results of studies on natural environments, but in evaluating such tests it seems desirable to define limiting factors in some way that will cause less confusion than the existing definitions are likely to create.

It is not impossible to define limiting factors in such a manner that

it can be applied to practical problems, but such a definition is almost certain to be touched with empiricism because any improvement of the existing laws must take into account the natural species variations in physiological reactions. It should be translatable into quantitative terms; it should be elastic enough to recognize that the effect of a limiting factor can be expressed in different ways at various stages in the growth cycle. Purely empirical definitions are suggested below.

Limiting factors—If a change in a particular factor or group of factors in an otherwise controlled environment produces an increase in the growth curve of the species or association in that environment, the factor or factors are said to be limiting. The degree of limitation can be expressed as the difference between the original growth curve and the maximum curve obtainable by changing the factors. For convenience the degree of limitation can be described in two ways. (a) *Absolute limitation*—The difference between the two populations at any particular time or stage in their growth cycles, and (b) *Relative limitation*—The difference between their relative growth rates.

As an example of the application of these definitions we may use the experiments in Figures 1 and 2. Figure 2 represents an experiment with natural sea water. Growth was much improved by adding fertilizer (Figure 1), and we conclude that one or more chemical factors were limiting in Experiment 2. With certain other physical conditions, the medium used in Experiment 1 produced a still larger growth, which indicates that no chemical factors were limiting in this experiment; in other words, growth was maximal for the particular physical environment. The degree of absolute limitation in Experiment 2, as indicated by the difference in population in the two experiments, was six million cells per liter at the end of the first day, twenty-six million the second day, fifty-six the third, and so on to a maximum absolute limitation of 448 million cells per liter at the respective peaks of the growth curves. The relative limitation, the difference in their relative growth rates, was .79 the first day, .76 the second, approximately zero from the third to the sixth, .27 on the seventh, .22 on the eighth day, and approximately zero through most of the subsequent decline in both populations. The advantage of expressing limitation in these two ways is that it not only states the difference in the amount of cells produced with a given change in the environment, but it also shows exactly when in the growth cycle the limiting effect is expressed.

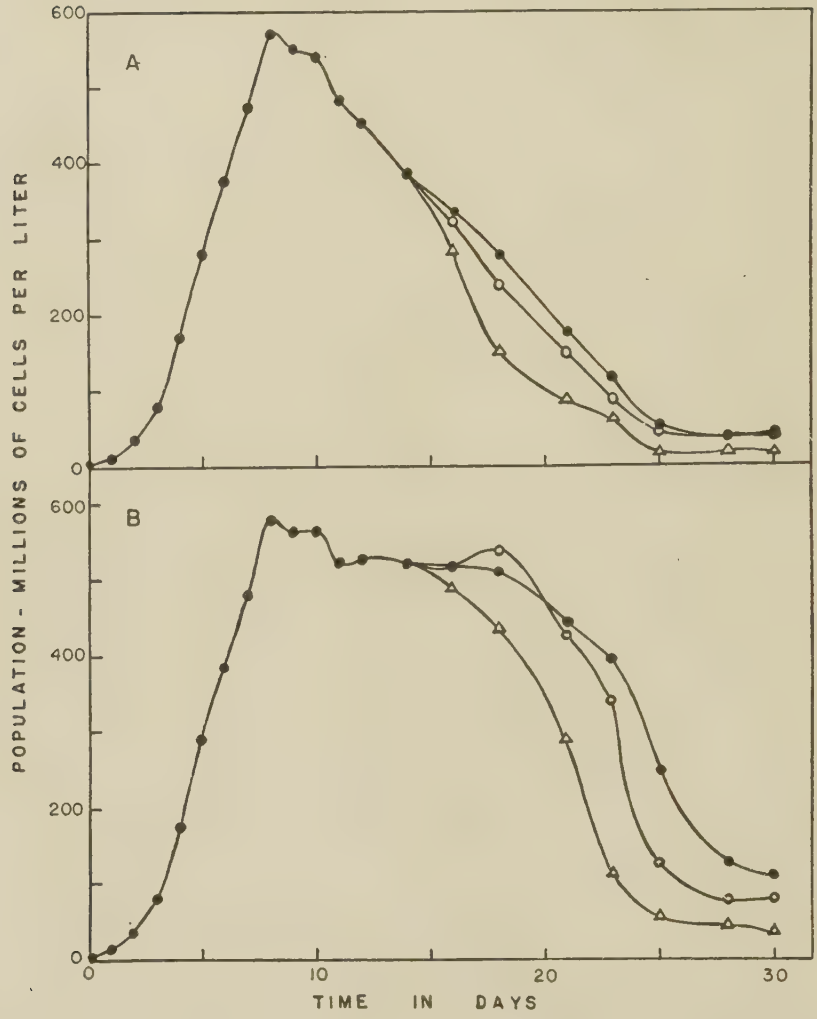
Senescence

Having discussed events leading to the peak of the growth curve, we now turn our attention to the subsequent decrease in population. Some of the events have already been described—the immediate decrease in active cells, the increase in senile cells, reaching a peak about the time when the active cells are decreasing most rapidly, and the subsequent decline of both. The appearance of these curves immediately suggests that active cells are being transformed into senile ones, and the latter are gradually dying, but this still leaves much to be desired in the way of an explanation.

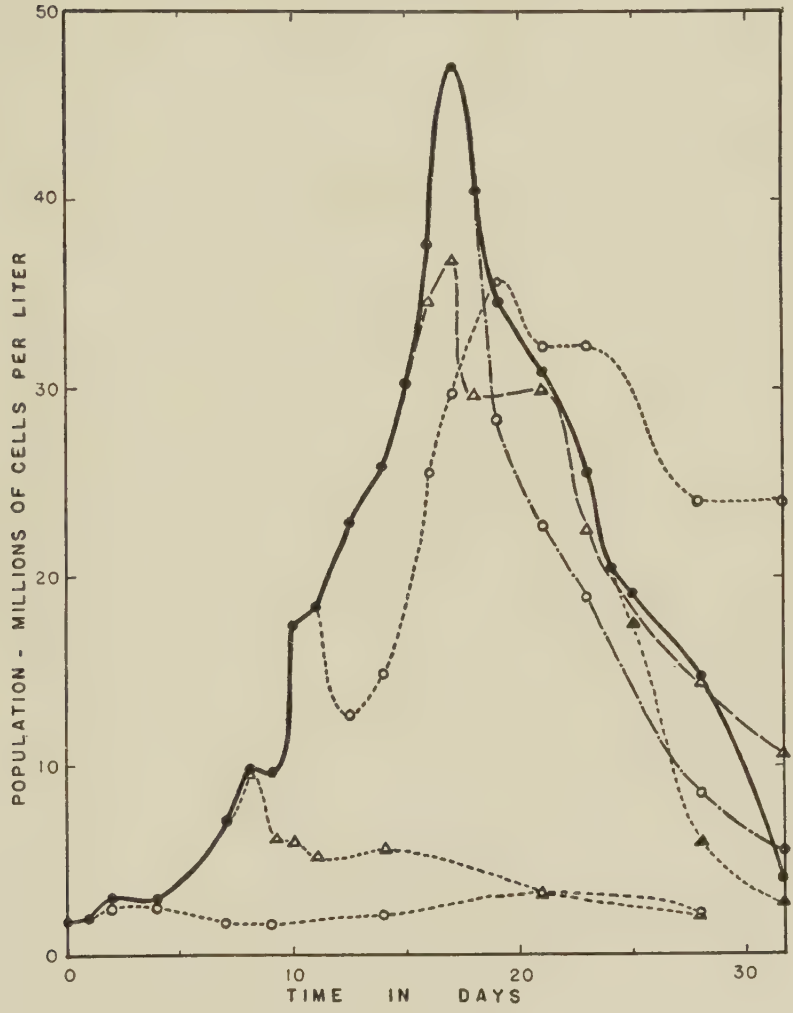
During the last part of the experiment in Figure 1, a subsidiary experiment was performed, the results of which are shown in Figure 10. The curves from Figure 1 are drawn again in Figure 10 (solid dots) with the active cells in 10A and the total population in 10B. A sample of the population was withdrawn to another flask and exposed to artificial light for only eight hours a day. This sample (circles in Figure 10) decreased somewhat more rapidly than the part of the experiment with continuous artificial illumination. A third sample (triangles) was brought up to room temperature, an increase of six or seven degrees, with natural illumination from a north window, which was superior to the second section of the experiment but probably inferior to the first. This sample decreased still more rapidly. Thus it appears that light somewhat inhibited the process of senescence, and an increase in temperature stimulated it. This is in line with the previous description in which it was shown that senescence is characterized by a decrease in the photosynthetic rate, which might be somewhat exaggerated by insufficient light, and by an increase in the respiratory rate, which would be brought about by a higher temperature.

Another experiment, which was designed for another purpose, yielded some information about senescence. Figure 11 (solid line) shows the growth curve of a Nitschia culture in natural sea water under constant illumination at a temperature of about 13°. At intervals small portions of the culture were drawn off and placed in the dark. The trends of these populations are shown in Figure 11 by various sorts of broken lines.

The two samples placed in the dark after the onset of senescence decreased somewhat more rapidly than the culture under continuous illumination, which is in accordance with previous conclusions. The



10. Senescence under varying environmental conditions. For complete explanation see text.



11. Effect on Nitschia cultures of storage in the dark. For explanation see text.

form of the curve was quite different, however, when the samples were drawn from an increasing population. In every case there was a further increase in the dark at a somewhat lower rate, generally beginning immediately, but in one experiment after a lag of a day and a half, and reaching a peak a day or a few days later. The populations then decreased, but at a slower rate than that of the culture exposed to light.

The initial increase in the dark was not entirely unexpected. It has been reported in laboratory cultures (Harvey, 1933) and in experiments with natural plankton associations (Riley, 1941). Presumably it is growth based on stored food reserves gained from previous photosynthetic activity. This is somewhat in accordance with the fact that the greatest subsequent growth in the dark was obtained at the point in the population curve where Figures 3 and 4 showed the photosynthetic rate to be highest.

The reasons for the later changes in the dark are not so obvious. The first sample removed from the culture appeared to decrease slightly and then increase again, and the population at the end of four weeks in the dark was about the same as in the beginning. The slight increase between the fourteenth and twenty-first days suggests the possibility that Nitschia may be able to grow saprophytically, though there is no further evidence in the rest of the experiments to support such a view. There were practically no typically senescent cells in this first culture, but after the first two weeks the majority looked rather abnormal, being slightly mis-shapen, with very thick cell walls. In the remainder of the samples there was a steadily increasing rate of population decline as well as an increasing number of cells that appeared senile. Thus senescence in the dark was related, directly or indirectly, to the physiological state of the culture or the magnitude of the growth that had taken place.

To propound a theory of senescence on the facts now available is largely speculative, although the facts fit into a logical framework. There are active cells present throughout practically the entire life cycle, in senescence as well as in active growth; they move and presumably carry on photosynthesis and multiply. Similarly, it has been noted that the so-called senile cells can be found in all stages of the life cycle. Obviously, then, when we speak of a culture being in a particular stage, we refer to the average condition of the culture, and in senescence we mean that an increasing number of cells, perhaps

most of them, are becoming senile, although it is apparent that some active cells have been transformed into senile ones during all the period of growth.

The mechanism of the gradual transformation of active cells into senile ones is thus related to the form of the growth curve, and some of the properties of the mechanism can be inferred from the results of experiments. The typical form of senescence is not caused primarily by depletion of the energy content of the cells, for cells of a young culture placed in the dark do not behave in that manner. The population decreases relatively slowly and shows little increase in the number of senile cells. The energy content is a secondary factor, however, which may, at a slightly later stage in the growth curve, contribute to the condition of senescence, as indicated by experiments in which cultures went into typical states of senescence due to decreases in illumination before the culture was depleted of nutrients. Either or both of these factors may initiate senescence, but generally it appears to be related to nutrient exhaustion. It has been noted, however, that senescence does not necessarily begin as soon as the nutrients are used up, but frequently some time later when further divisions have reduced the nutrient content of the cells to a small percentage of its normal value. This suggests the possibility of a minimum limit for the nutrient content of the cells, and that through successive divisions they approach this limit and even go past it, so that they are no longer able to carry on an active life and begin slowly to die.

If this latter point is true it resolves the matter into a species characteristic, or perhaps a characteristic of diatoms in general, to continue division beyond the "safety" point as distinguished from organisms that have more rigid nutrient requirements, so that the nutrients freed from the organisms by death are utilized in further growth, with the result that growth balances death and the population approaches an asymptote.

The Nitschia mechanism appears at first glance a poor one from the standpoint of race survival. Actually, however, in the peculiar environment of pelagic diatoms it is so much superior that the author feels it may have evolutionary significance. This is due to a combination of two factors. In the first place, some few of the senescent cells persist in a viable condition for weeks or even (according to Allen and Nelson) for years, so that they are in effect resting cells of a primitive

kind. In the second place, as will be shown later, they sink more slowly than active cells, a factor of the greatest importance in maintaining a pelagic population in the surface waters through a period of unfavorable growing conditions.

Subsidiary Experiments on Nitschia

A few other experiments were performed for various reasons—to aid the explanation of the behavior of experiments with natural associations, or to explore the possibilities of alteration in growth under special conditions that might be obtained in the natural environment.

One of these experiments has already been discussed in some detail and shown in Figure 11. It was prompted by two questions. The first concerned the fate of cells that sink below the euphotic zone, how long they could remain in a viable condition and what form of curve such a population might have. The experiment did not give a single answer to the question. It showed that such cells, if derived from a population in the stage of active growth, might increase in number at first and then decrease slowly, or, at later stages of the growth curve, might decrease rather rapidly. In either case such cells might persist in a fairly active or at least viable condition for several weeks, and either the increase in the number of cells or the gradual dissipation of their energy content might be expected to lower their specific gravity, thus decreasing the sinking rate and leading to a concentration of the population in the region of the discontinuity layer, such as has been noted many times.

The second question concerned the behavior of natural populations in experimental dark bottles, which sometimes increased and in other cases decreased. A comparison with laboratory experiments was needed to determine whether this behavior was characteristic of diatom cultures or was due to special influences of the more complex natural environment. The Nitschia experiment shows these phenomena to be entirely possible; the detailed comparison, however, is reserved for a later section.

Other experiments were performed to determine the sinking rate at various stages of growth and to explore the possibilities of modifying the growth curve by removing part of the population or by supplying additional nutrients.

The Sinking Rate

The sinking rate was determined as follows: The culture was shaken thoroughly to insure uniform suspension of the cells, and one milliliter was plated out in a Sedgwick-Rafter cell with a depth of 1 mm. At intervals of a minute or a few minutes counts were made of the number of Nitschia cells collecting on a particular area of the bottom of the cell. This was continued until there was no further increase in number, indicating that all the cells that had been in suspension had come to rest. The distance traveled by each cell was obviously 0 to 1 mm., averaging, in a uniform suspension, 0.5 mm., and the mean sinking rate for this distance was the average time that the cells required to appear on the bottom. The method is incomplete since it does not permit accurate measurement of individual variations in sinking rate, but it is an extremely simple way of arriving at the mean.

That such individual differences do exist was shown by the fact that the number of cells appearing on the bottom per unit of time decreased toward the end of each experiment instead of remaining constant throughout. The variation appeared to be rather slight as far as the actively growing cells were concerned, but was very marked in senescent cultures.

The experiment in which the sinking rate was determined has been shown in Figure 9 (triangles and broken line). The sinking rate at various stages of the culture is shown in Table II, together with measurements showing that the observed changes were not due to a progressive change in the size of the cells.

The sinking rate of active cells may have been slightly greater at first, but was fairly constant thereafter. That of senile cells was strikingly less, and the difference, as indicated in a previous section, is considered to be singificant enough to have survival value in the natural environment. The difference is due in part to the decrease in the organic matter content of the senile cells. The shape, though not the size, of the cells may also be important, for in senescence many of the cells are curved at the ends, which orients them so that the broadest surface is always horizontal, as contrasted with straight ones, which turn end over end and undoubtedly sink faster when they are vertical. Just how important this factor may be has not been determined although rough tests indicate that it is secondary to the organic content.

Table II. Sinking Rate and Cell Size

Age of culture in days	4	6	9	13	20	30
Population (millions per liter)						
Active cells	8.3	23.3	45.8	94.5	50.4	17.1
Senile cells	.5	1.6	3.3	12.4	29.6	7.8
Total cells	8.8	24.9	49.1	106.9	80.0	24.9
Total length of cells (μ)	34.2	34.1	33.8	33.9	34.0	33.8
Length of cell body (μ)	13.7	14.3	13.3	13.6	13.8	13.6
Width of cell body (μ)	3.2	3.2	3.0	3.2	3.0	3.1
Sinking rate (mm. per day)						
Active cells	63	52	50	53	50	48
Senile cells	—	—	—	42	22	25
Total cells	63	52	50	51	31	35

Another factor of great importance, noted in most of the experiments, is the fact that near the peak of the population the cells tended to agglutinate and to sink much more rapidly than when they were in uniform suspension. Such aggregates stuck together with great firmness, and appeared to be imbedded in or stuck to masses of detritus. The work of Ohle (1935), who experimented with the effect of pH on the aggregation of organic matter, prompted the author to perform one rough experiment on the subject. A culture just past the peak and beginning to agglutinate badly was divided into two parts. One flask was saturated with carbon dioxide and both were shaken thoroughly and placed in the dark for six hours. At the end of that time a sample was removed from a point midway between the surface and bottom of each flask with considerable care to prevent disturbance of the contents. The CO_2-treated flask had 50% of its population still in suspension; the control 30%. The experiment is provocative but in itself proves little, and other work has prevented pursuing the matter further.

Removal of Part of the Culture and Periodic Enrichment

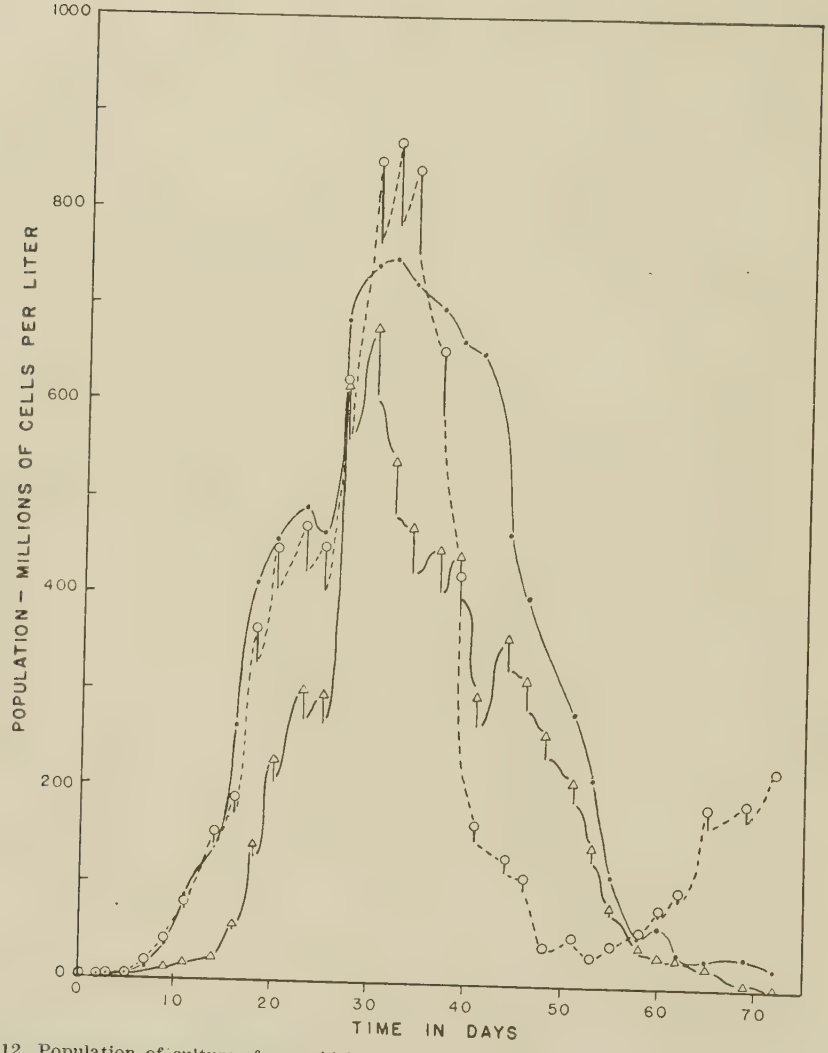
There are certain processes in the natural environment which remove part of the phytoplankton population from the euphotic zone, and might therefore be expected to alter any tendency toward the establishment of a typical growth cycle in nature. One of these is vertical turbulence. It removes part of the water and the population growing in it and replaces it with water that generally contains little plankton but has abundant nutrients. Two others, zooplankton feeding and

the natural sinking process, remove some of the population but have little or no effect on the chemical environment.

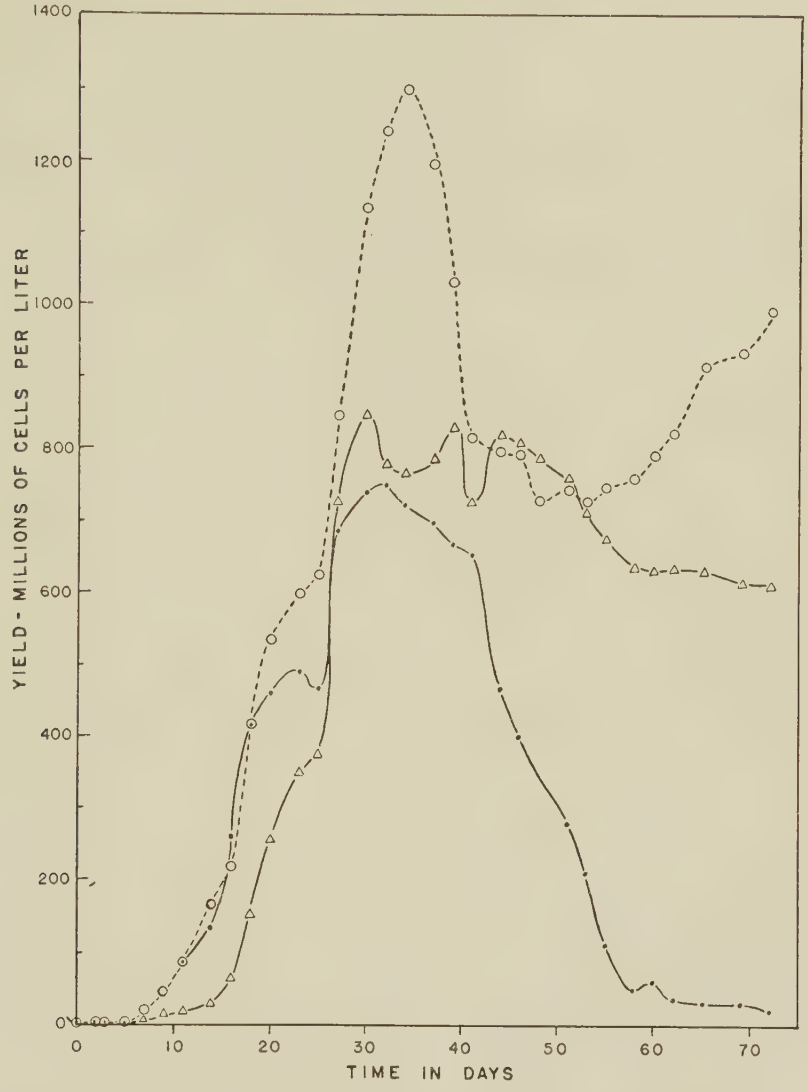
These processes are easily simulated in the laboratory. Three flasks were set up containing aliquots of the same culture medium and seeded with equal numbers of Nitschia. One culture was allowed to develop normally (dots and solid line in Figure 12). From the second (circles) one-tenth of the culture was removed every two or three days and was replaced by an equal quantity of the original culture medium. From the third flask (triangles) a tenth of the culture was taken out every two or three days and filtered to remove the Nitschia, and the filtrate was returned to the flask. The reduction of the population by removal and dilution is indicated in Figure 12 by the vertical lines extending down from each point in the second and third parts of the experiment.

Ketchum and Redfield (1938) have shown that it is possible to maintain a continuous, constant, and rapidly growing culture by removing the daily yield and replacing it with fresh medium. Under the less carefully regulated conditions of the present experiment, this did not occur. In the beginning, the effect of enrichment was to increase the growth rate so that the culture kept pace with the control in spite of the periodic dilution of the population. It also achieved a peak at a slightly higher level, and the total yield (the population plus the total number of cells removed), shown in Figure 13, was markedly greater. But having reached a point where the increase during a two-day period just matched the amount removed, the culture did not long maintain that level; rather, it went into a state of senescence four days later, during which there was no evidence that enrichment had any beneficial effect. It is true that the culture recovered quickly and was on the upgrade at the time the experiment was concluded, but the secondary increase was subsequent to a very pronounced period of senescence.

The culture in which part of the population was removed, but with no enrichment, increased somewhat more slowly than the other two and came to a peak at a lower level, although the total yield was considerably greater than in the control. The most interesting point to be noted here is that the Nitschia culture would be able to offer a considerable quantity of food to a zooplankton population (a tenth of itself every two or three days and totaling in two months an amount nearly as great as its peak population) without any very striking alteration of the form or size of the growth curve.



12. Population of cultures from which part of the population was periodically removed and replaced with fresh medium (circles) or the same medium with cells removed (triangles). Dots represent control.



13. Total yield of cultures from which part of the population was periodically removed and replaced with fresh medium or the same medium with cells removed. Symbols same as in Fig. 12.

EXPERIMENTS ON NATURAL PLANKTON ASSOCIATIONS

Four cruises to Georges Bank during the spring of 1941, although largely occupied with routine analyses, provided opportunity for work on the behavior of the natural plankton associations. Eleven experiments were performed to determine something of the rates of photosynthesis and respiration, of increases in cell number and plant pigment content, and of phosphate and nitrate consumption, before, during, and after the spring diatom flowering. In addition to these, three experiments were performed on the sinking rate.

Productivity Experiments

During the 1940 cruises (Riley, 1941a), the photosynthetic and respiratory rates of the Georges Bank plankton were determined by exposing light and dark bottles of surface water for twenty-four hours in a tub on the deck of the "Atlantis" and subsequently analyzing for oxygen. The method appeared to be satisfactory, and the results proved useful in estimating the phytoplankton productivity of Georges Bank, but they suggested the desirability of more detailed information. Accordingly, the experimental technique in the following year was limited to a smaller number of experiments in which more analyses were made—plant pigments, cell counts, and P and N determinations in addition to the oxygen titration. The only changes in the method were the use of larger bottles and the extension of the length of exposure from twenty-four hours to several days (two to four). The latter modification was unfortunate because it involved some errors (such as an unnatural increase in bacterial numbers), but it was necessary since some of the methods used were too inaccurate to yield significant results in a twenty-four-hour period.

During the first cruise, in the latter third of March, three experiments were performed, all of which represented conditions just prior to the beginning of the spring flowering. Four experiments in the middle of April showed the period of rapid expansion of the flowering, and two in early May and two in late May illustrated senescent stages.

Analyses of the experiments are summarized in Table III. Columns 5 and 6, the quantity of plant pigments at the beginning of the experiment and the cell number respectively, are closely related (r, the coefficient of correlation, is .962). It is gratifying to note that under certain circumstances the results of these two methods may be com-

TABLE III. PRODUCTIVITY EXPERIMENTS ON GEORGES BANK

Station No.	*Date*	*Position Lat.*	*Long.*	*Initial plant pigments*	*Initial cell number*	*Oxygen production*	*Oxygen consump-*	*Plant pigment production*	*Plant pigment consumption*	*Cell production*	*Cell consumption*	*P cons.*	*N cons.*
4178	3/21/41	41–21	68–19	7200	21,700	.00	.00	350	450	−50	5100	.00	.4
4203	3/29/41	41–48	67–22	8400	12,300	.11	.04	1300	−1000	5400	1250	.00	.5
4205	3/30/41	41–52	66–51	8200	5500	.06	.11	500	1000	810	120	.01	1.3
4211	4/15/41	41–21	68–19	54,100	683,000	.56	.19	2600	−2000	168,000	−79000	.11	1.1
4220	4/17/41	41–17	67–37	14,500	60,600	.12	.11	2800	1600	20,300	−5900	.06	.0
4232	4/19/41	41–39	66–35	12,500	138,300	.37	.20	8000	−300	136,400	−2800	.22	.6
4240	4/22/41	41–44	68–16	46,500	773,000	.64	.12	2300	−2700	222,000	−14000	.06	.2
4244	5/7/41	40–55	68–36	29,600	413,000	.65	.33	5600	−2400	47,000	700	.08	.1
4263	5/10/41	41–53	67–22	21,600	398,000	.42	.15	800	−700	−2700	32,000	.07	.1
4279	5/28/41	41–21	68–20	8300	7200	.07	.22	2000	1100	2400	1500	.03	.0
4289	5/30 41	41–18	67–37	13,600	25,600	.20	.14	30	800	3200	1300	.01	.1

Plant pigments are presented as "Harvey units" per m^3, cells as number per liter, oxygen as mgs. per liter per day, production and consumption of plant pigments and cells as units per day, and P and N as mg.-atoms per m^3 per day.

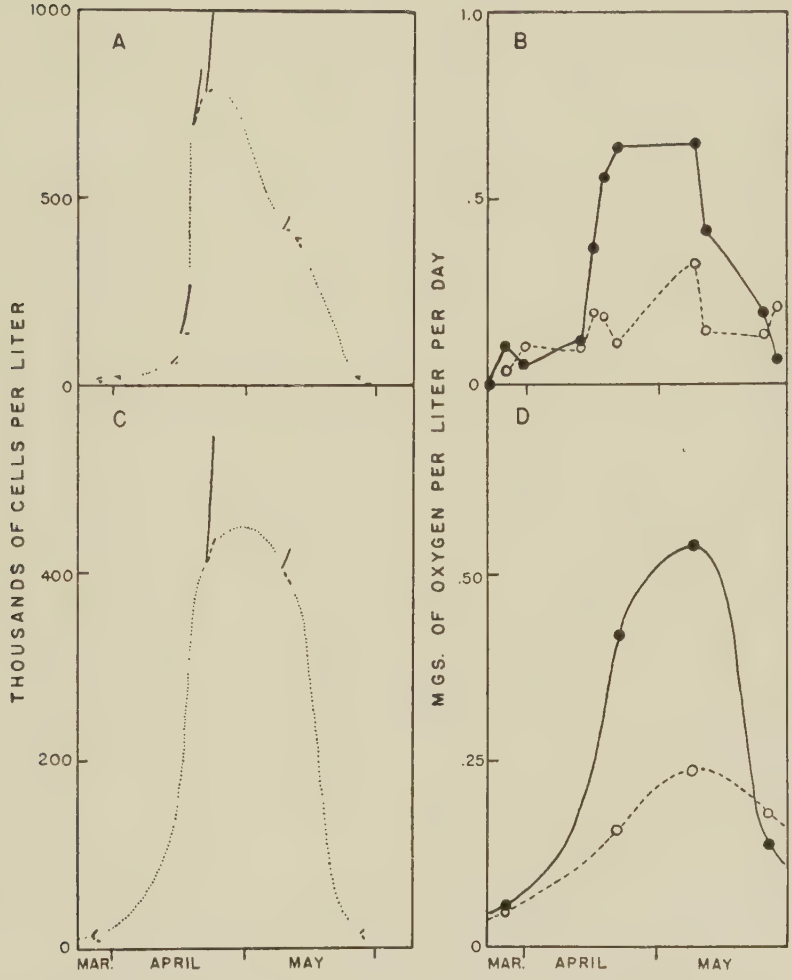
parable and more or less interchangeable. Such was not the case in measurements of net plankton made the previous year. The quantities of plankton are also significantly related to certain of the processes occurring in the experimental bottles. There is a correlation of .852 between plant pigments and oxygen production, and of .904 between cell number and oxygen production. The correlation of initial cell number with cell production is also significant (.795), although the corresponding plant pigment relationship is not.

Some of the productive processes in the experimental bottles are also interrelated. Most important of these are: oxygen production and cell production (.726), oxygen production and phosphate consumption (.540), plant pigment production and phosphate consumption (.871), and cell production and phosphate consumption (.574). Nitrate consumption shows no significant correlations with other experimental values, but this may be due to the fact that the analytical technique is hardly accurate enough to permit good determinations of the small differences obtained during the experiments.

The reactions in the dark bottle are somewhat complicated. The relationships of oxygen consumption with the initial plant pigments and cell number are positive but not very significant (.348 and .340, respectively). As in previous experiments (cf. Riley, 1941), the plant pigments in the dark bottle decreased in some cases and increased in others. The increase was most marked when the initial plant pigment content was high, and the correlation between initial plant pigments and plant pigment consumption was $-.788$. The relationship between cell number and cell consumption in the dark bottle tended to be similar, but the correlation was only $-.498$.

In the paper referred to above, the experimental data were subjected to extensive statistical treatment which showed that such processes as photosynthesis, nutrient consumption, and plant pigment production normally occur in direct sequence, but are discrete processes that are separable under certain environmental conditions that tend to inhibit one or more of them.

The present work makes no attempt to carry the statistical analysis to a higher level, for the quantity of data is not sufficient to warrant it. It is merely noted in passing that the simple correlations indicate, in so far as such a treatment is able to do, a type of relations similar to those obtained in more detailed studies. In another respect, however, this small group of experiments can supplement previous findings, for

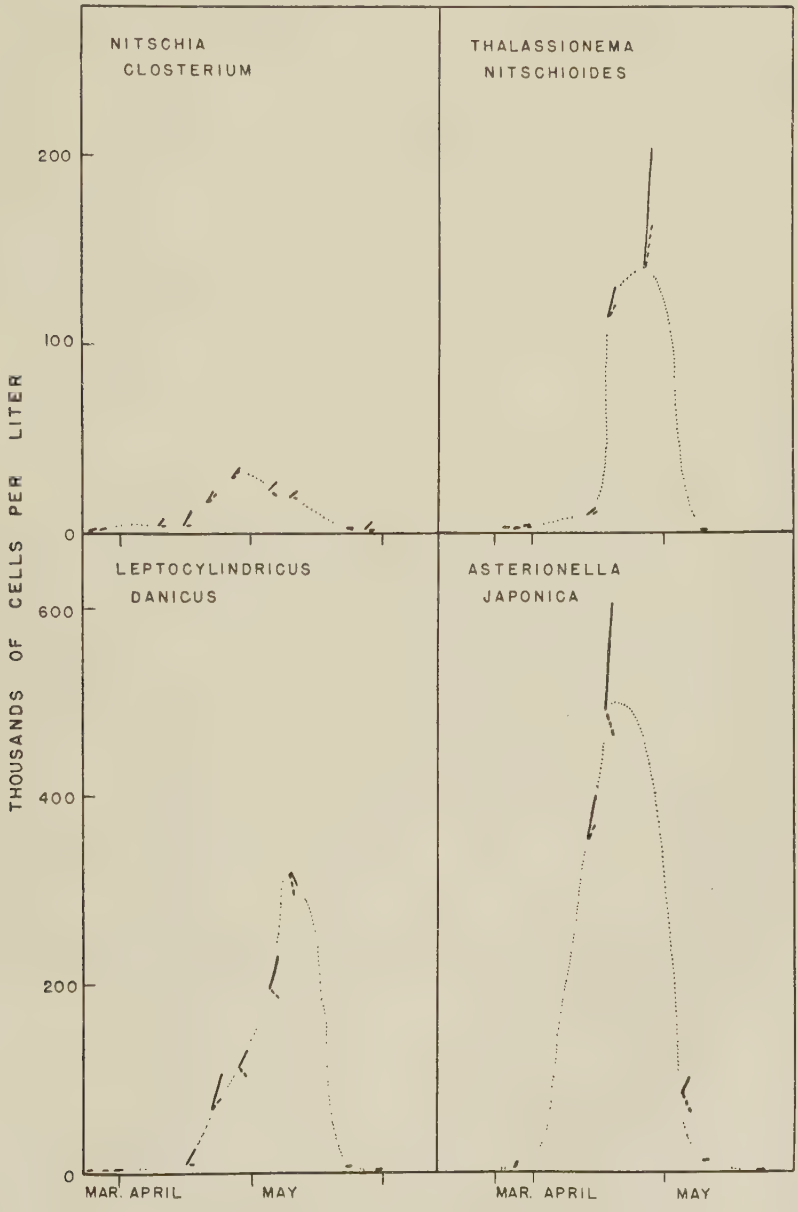


14. Diatom production, photosynthesis and respiration of Georges Bank plankton. For explanation see text.

it combines experimental values with species determinations in such a way as to permit comparison with the simpler results obtained in experiments with pure cultures. This is especially true since the Georges Bank experiments covered the period from the beginning to the end of the spring diatom flowering, the part of the seasonal cycle that one might expect to most nearly approximate a laboratory growth cycle.

For an initial comparison the experimental results for each cruise were averaged. These included three experiments in late March, four in the latter half of April, two in early May, and two in late May. In Figure 14C the averages of the values for initial cell number are plotted and connected with a broken line that idealizes the general form of the spring diatom flowering. Superimposed on the curve are the experimental values for the daily increase of cells in the light bottle (solid lines) and the change in cell number in the dark bottle (dotted lines). In Figure 14D are plotted the experimental determinations of oxygen production (solid line) and oxygen consumption (circles and dotted line). It is apparent from comparison with previous figures that there is considerable resemblance to some of the Nitschia curves, particularly in the general form of the curves for oxygen production and consumption, the magnitude of the rate of growth in relation to the stage of growth (as indicated by light bottle experiments), and the form of the change occurring in the dark bottle. In one respect the results differ markedly from laboratory cultures, namely that the rate of growth in experimental bottles is much greater than the apparent growth of the natural association. This, however, is readily understandable since not only does the experiment have better light than have the Georges Bank plankton as a whole, but also it is subject to no losses by sinking.

But however close such a comparison may be, it is defective in several respects. A glance at Table III will show that the quantity of plankton varied greatly during the course of a single cruise, particularly in April. A cruise average therefore does not represent a single stage of the growth cycle but a composite of several stages. Whether or not this is a serious error depends on how widely varied the different stages are, and to attempt to avoid such an error by the use of individual station records resolves itself into a dilemma. One method is to arrange the experiments chronologically, but this is obviously a mistake because the spring flowering does not begin at the



15. Georges Bank diatom production. See text for explanation.

same time all over the bank or develop at the same rate (Riley, 1942). Only by drifting with a particular mass of water and sampling it at regular intervals could a true chronological record be obtained. Another way out of the difficulty would be to arrange the experiments in a roughly chronological order, that is, with the data of each cruise together, but with the individual experiments within the cruises arbitrarily placed according to their proper position on a hypothetical curve representing the increase and decline of the spring diatom flowering. This is done in Figures 14A and B, and to some extent it enhances the comparison with the Nitschia experiments; for example, in 14A compare the changes in the light (solid lines) and in the dark (dotted lines) with similar changes in the Nitschia culture shown in Figure 11. The comparison is probably valid since the trends in the experimental bottles are somewhat similar to the postulated curve for the spring flowering, but it should be regarded as hypothetical because of the arbitrary element of arrangement.

Another objection is the fact, or at least the probability, that not all species in the association are in the same stage of growth. While deserving consideration, this objection probably has less validity during the spring flowering than at any other time of the year, for then a few species constitute the major part of the association, and they reach their peak at about the same time. The best solution is to pick out and subject to separate treatment certain species that are present in sufficient quantities to permit accurate counts in all, or nearly all, of the experiments. This is done in Figure 15 for four species, according to the same plan used in Figure 14A. It is quite clear from the figure that species from the natural environment can be fitted with curves that are quite similar to the laboratory results, and that the results of the field experiments show the same general trend as the suggested growth cycle, except that in many cases species were capable of increasing in the light bottle after they had reached a senescent stage in the natural waters.

Sinking Rate

During each of the three latter cruises to Georges Bank in the spring of 1941, a rough measurement was made of the sinking rate of the surface plankton at one station. On as calm a day as possible, a sample of surface water was taken with a bucket, of which 200 ml. was preserved for counting, and a liter of the remainder was poured

into a graduated cylinder 35 cm. long. After standing five hours, the water in the cylinder was slowly siphoned off with a small rubber tube that was held so that its opening was about 1 cm. below the surface of the water in the cylinder. The first 200 ml. and the middle 200 (from the 400 to the 600 mark on the cylinder) were collected for counts. When only 200 ml. remained, the tube was removed, and the graduate was shaken thoroughly to resuspend any organisms that had settled to the bottom, and the water was poured off and preserved. The counts on the initial population and the upper, middle, and lower water in the graduate are shown in Table IV.

TABLE IV. SINKING RATE OF GEORGES BANK PLANKTON

	Genera or species	*A*	*B*	*C*	*D*
Station 4240	*Asterionella japonica*	485,000	270,000	332,000	845,000
4/22/41	Thalassiosira spp.	41,000	30,000	42,000	177,000
	Chaetoceros spp.	95,000	72,000	83,000	79,000
	Leptocylindricus danicus	40,000	30,000	50,000	52,000
	Thalassionema Nitschioides	42,000	30,000	50,000	147,000
	Rhizosolenia alata	3000	1000	—	1000
	Rh. hebetata	1000	1000	1000	—
	Coscinodiscus spp.	4000	1000	—	1000
	Total	**711,000**	**428,000**	**561,000**	**1,302,000**
Station 4273	*Leptocylindricus danicus*	86,900	51,500	49,700	235,500
5/13/41	*Nitschia closterium*	6900	3200	3700	15,300
	Thalassiosira spp.	5100	12,000	5100	6100
	Navicula sp.	1800	900	—	800
	Chaetoceros spp.	20,700	14,300	18,400	39,100
	Asterionella japonica	8700	—	2300	33,700
	Rhizosolenia hebetata	1800	900	—	7700
	Thalassionema Nitschioides	3700	—	1400	3100
	Total	**135,600**	**82,800**	**80,600**	**341,300**
Station 4289	Peridinians	9000	19,400	1800	10,800
5/30/41	Rhizosolenia sp.	5100	700	2800	16,600
	Chaetoceros spp.	4100	4400	2600	2600
	Nitschia closterium	500	200	—	1000
	Navicula sp.	100	100	—	—
	Leptocylindricus danicus	6000	3700	4000	3700
	Thalassiosira spp.	800	100	200	300
	Navicula Vanhoeffini	—	—	—	100
	Coscinodiscus spp.	—	—	—	600
	Total	**28,600**	**28,600**	**11,400**	**35,600**
	Total diatoms	**16,600**	**9100**	**9600**	**24,900**

A. Count of initial sample; B. Upper two hundred milliliters of water in graduate after standing five hours; C. Middle two hundred; D. Lower two hundred.

The relative quantity of diatoms remaining in the upper 200 ml. of the experiment after five hours varied little in the three experiments, ranging from 55% to 61% of the original quantity. In two of the three experiments the quantity of diatoms in the middle sample was about the same as at the surface, and in no case was the difference as great as that between the middle and lower samples. This suggests that part of the diatoms remained fairly uniformly suspended, while the remainder sank rather rapidly.

There is some slight indication that individual species tended to sink more rapidly just at the time when they reached the peak of their growth curve than either before or afterward. In seven major species or genera the quantity of diatoms in the bottom sample averaged six times the surface sample when each species was at or near its peak. Before and after the peak the average was one and a half times the surface value. The probability that such a result might be obtained by chance is about 1:10 (according to the method of determining the significance of the difference between means of small samples by values of *t*). Statistically speaking, this is on the borderline of significance, favoring the hypothesis but not adequate proof. But since a tendency toward rapid sinking was noted at the peak of the Nitschia cultures due to clumping of the cells, the results suggest the possibility of a similar phenomenon in the ocean, which, if true, would be an important biological factor in spring diatom flowerings and is certainly worthy of further investigation.

The distribution of peridinians was quite different from that of the diatoms, nearly all of them being in the upper or lower sample. This suggests that some of them were able to maintain a surface position more adequately than diatoms, probably by active swimming motions, since the sinking rate of preserved specimens was rapid. Whether the ones at the bottom were in poor condition or were simply negatively geotropic was not determined.

GENERAL DISCUSSION

The curve for almost any spring diatom flowering resembles the sort of growth curve that may be seen in a pure diatom culture in the laboratory. Comparison of the two involves certain arbitrary elements because of the lack of homogeneity in natural populations; nevertheless the validity of the comparison has been strengthened by

examining the growth curves in the two environments in more detail—in the curves for individual species, in the rates of change of natural populations imprisoned in experimental bottles, and in the form of the curves for photosynthesis and respiration. The discussion proceeds, therefore, on the assumption, at least partly warranted, that at the time of the spring diatom flowering the major species pass through a growth cycle somewhat similar to that observed in pure cultures, except in so far as it is modified by special environmental factors. In attempting to apply the knowledge gained from the study of Nitschia cultures to spring diatom flowerings, two main points are considered: first, how certain aspects of the normal growth curve can be used to explain field problems, and second, how the variability of the natural environment affects the normal diatom physiology.

The Initiation of the Spring Flowering

A previous paper (Riley, 1942) has shown that the inception of the spring flowering can be correlated with physical factors of the environment: the vernal increase in solar radiation and the degree of vertical turbulence. The hypothesis of the interaction between these two physical factors explained observed correlations between solar radiation and the time of the spring flowering, and it also showed why the diatom burst began earliest in shallow or highly stable areas, even as early as January in certain bays and sounds.

While the theory fit the facts as observed in analyses of Georges Bank waters, the experimental data now available indicate that physical factors do not provide a complete explanation. If the only thing required to start the flowering were that the diatoms be assured ample radiation by being constrained to a shallow surface stratum, then one would expect an immediate growth in the experimental bottles. Inspection of the March experiments shows that this did not occur. It was not until April that such increases were observed, when the diatoms were also growing rapidly in the natural waters. But it has been seen in the Nitschia experiments that physical conditions adequate for growth are not the only prerequisites, that there is frequently a lag period during which the population remains stable or increases very slowly.

Such a lag may, and probably does, occur in natural diatom populations as well. If so, it would explain the failure of the March experiments to exhibit immediate growth. This does not invalidate the

theory of the importance of physical factors, since obviously they must achieve a certain degree of adequacy before any pronounced growth can occur, but the theory is modified to the extent of introducing a lag between the time when they become adequate and the time when the effect is realized. Unfortunately, however, it is impossible to determine from any data available just what the radiation threshhold or the length of such a lag period might be.

The Peak and Decline of the Spring Flowering

Analyses and experiments indicate that during the earlier stages of a diatom flowering, growth proceeds somewhat more slowly than in enclosed bottles of surface water or in laboratory experiments (presumably because the illumination of the population as a whole is not nearly so adequate), but the increase takes a form approximating geometrical progression. What brings the growth of the population to a stop is a difficult problem, in which there are several possibilities, little unanimity of opinion, and a scarcity of data that permit quantitative evaluation of factors.

One rather clear case has been presented by Harvey, Cooper, Lebour, and Russell (1935) to show that in the English Channel the diatom population is controlled by zooplankton grazing. Later Fleming (1939) worked out the mathematical theory of the process, based on assumptions that the diatoms grow at a uniform rate and are fed upon by a steadily increasing number of animals. The form of the diatom population curve so obtained is bell-shaped and symmetrical, and it fits the English Channel data in a satisfactory way.

Quite a different situation was observed in Long Island Sound (Riley, 1941). There, as in many other places, the diatom flowering was accompanied by an increase in zooplankton, but the phytoplankton-zooplankton ratio was far higher than in the English Channel. A statistical estimate of zooplankton feeding suggested that in the winter and early spring when the flowering came to its greatest height, the food requirement was about eighteen per cent of the animals' weight per day, or about one per cent of the phytoplankton crop. Even if feeding reached fifty per cent of the animals' weight, as suggested by Harvey *et al.* the reduction of phytoplankton would be relatively insignificant.

Just how the limitation was effected in Long Island Sound is uncertain. It is known that nitrate and phosphate, particularly the latter,

reached a fairly low level; moreover the transparency was greatly reduced. These factors have all been shown experimentally to be important.

Without solving this latter problem completely, it is still apparent that diatom flowerings can be controlled either by grazing or by the interaction of the diatoms with their chemical and physical environment. Probably there are few areas in which the separation of factors is as complete as in these two extreme cases. On and near Georges Bank, and perhaps in many oceanic areas, the phytoplankton-zooplankton ratio is intermediate between Long Island Sound and English Channel measurements. Presumably on Georges Bank the zooplankton is at least partly responsible for the observed changes in the plant population, but there is also evidence that limitation of growth might be effected by the reduction of the transparency of the water.

Obviously any one or any combination of these factors could reduce the growth rate of the population and lead eventually to the dissolution of the spring flowering. The theoretical distinction between the two types of limitation is whether the growth rate expressed in terms of true productivity remains logarithmic (in the case of grazing control) or declines to a point below the death rate (chemical or physical limitation). This appears to be the proper experimental approach to the problem. In any case the forms of the population curves would vary so little from the typical symmetrical form that any of the theoretical types could be fitted equally well by the data from a diatom flowering and consequently could not be used to identify the phenomenon involved.

Other evidence can sometimes be detected in the raw data, although it is never of a critical sort except in extreme cases. Any area such as the English Channel, in which diatoms are largely controlled by grazing, is readily revealed by the paucity of phytoplankton at lower levels. Conversely, lack of such control is revealed by accumulations of diatoms near the bottom or at some intermediate level, but here there is no proof that the zooplankton population is not also important. The latter is generally judged by numerical abundance and relation to the form of the phytoplankton curve, both of which are by themselves somewhat indefinite criteria. Thus, in the Georges Bank data, preliminary statistical treatment has revealed significant negative relations between zooplankton and phytoplankton. These permit a

certain amount of quantitative evaluation of the grazing factor, which will be presented in a subsequent paper. The vertical curves for phytoplankton are indicative also that the crop is far from completely consumed before it reaches the bottom.

In early May, after the peak of the flowering, there is almost universally present a sparse surface crop, much of which looks rather debilitated, and a much larger population at some lower level. The latter suggests that sinking is an important phenomenon. This is especially interesting in view of the suggestion from a previous section that a certain part of the Georges Bank plankton begins to sink rapidly just at the time when the population reaches its peak. According to the Nitschia experiments, this points toward at least partial control of the flowering by physical or chemical means. However, the fact that the plankton increased in some of the light bottle experiments in early May indicates that it was not in a typically senescent condition. Probably growth was either temporarily limited by light, as in some of the experiments shown in Figure 9, or was approaching senescence and still growing slowly but being subjected to increasing inroads by grazing zooplankton.

The phytoplankton left in the surface waters after the end of the flowering is worthy of brief mention. As stated before, many of the diatoms had a senile appearance, but some appeared to be in good condition. Experiments showed that some species were capable of quite active growth while others decreased in experimental bottles. It is evident that conditions for growth were not so favorable as they had been earlier in the season, but this could not account completely for the scarcity of diatoms. The explanation presumably lies in part in the grazing phenomenon, and the dominance of small species suggests selective feeding. Also probably the ability of some of the dinoflagellates to keep themselves at or near the surface, as shown in experiments, is a serious factor in the competition for available nutrients, for the diatoms, with a fixed sinking rate and with continual losses of part of their population from this cause, would have to multiply more rapidly than the dinoflagellates in order to maintain their population level.

SUMMARY

1. Laboratory experiments on the growth of the diatom, *Nitschia closterium*, were combined with experiments on the natural plankton associations of Georges Bank in an attempt to describe some of the physiological factors involved in spring diatom flowerings.

2. The growth curve of Nitschia was described according to the following phases: an initial lag, a period of logarithmic growth, a period of decreased growth rate with the population reaching a peak, and a subsequent decrease to a low population level.

a.) The initial lag was not always present. It was largely dependent on the size of the initial population, being longer when the original quantity was small. The quality of the environment played a minor role.

b.) The period of logarithmic growth took place at a rate which was fairly constant for a particular physical environment. The length of this period appeared to be determined by the adequacy of the chemical environment.

c.) The subsequent decrease in growth rate appeared to be due largely to exhaustion of nutrients, although decreased light intensity, either external or internal (decreased transparency of the culture) produced the same effect. A discussion was presented of the laws of limiting factors.

3. Coincident with the decrease in the growth rate of the population, there was an increase in the number of so-called "senile" cells, which were relatively inactive and readily distinguishable morphologically from active cells. The number of these cells reached its peak at the time when the culture was decreasing most rapidly. Many of them died later but a few remained in a viable though inactive state, so that some of the senescent cultures achieved a low but relatively constant population which could be activated again by improving the chemical environment. Theories are suggested to account for the transformation of active into "senile" cells and to explain their ecological significance.

4. The curve for photosynthetic activity in the Nitschia cultures followed the growth rate curve, reaching its maximum height at the time when the population was growing most rapidly. The respiration rate remained fairly low during this period but reached a maximum later, when the population was decreasing. It is not clear whether

the latter was attributable to the Nitschia cells or was due to bacterial activity.

5. Removal of part of the culture at regular intervals and replacement with fresh culture medium or with the same medium with the cells filtered out caused slight increases or decreases, respectively, in the population curves, but the latter were not altered significantly in their general form.

6. When parts of a Nitschia culture were placed in the dark, the subsequent events depended on the stage of the culture from which the sample was derived. When it came from a young, actively growing culture, there was a considerable increase in the dark, followed by a gradual decrease that was much slower than that of the usual senescent culture. When the sample was drawn from a culture past its peak, the change was the opposite, a more rapid decrease in the dark than in the light.

7. The sinking rate of normal Nitschia cells was found to be of the order of 50 mm. per day. Senile cells sank about half as rapidly as active ones, but they tended to agglutinate into masses when the culture was near its peak, and these masses of cells sank very rapidly. There was some evidence of a similar tendency on the part of the natural plankton population of Georges Bank.

8. Experiments on the Georges Bank plankton during the spring flowering suggested that the dominant species passed through a growth cycle similar to that of the Nitschia experiments. Experiments with natural plankton in light and dark bottles showed certain features common to both. The curves for photosynthetic and respiratory activity and the rates of increase in light and dark bottles in relation to the stage of the growth curve were important in this respect.

9. A general discussion is presented of physiological factors involved in spring flowerings, particularly a lag period preceding the flowering, the effect of the physical and chemical environment in limiting growth, and the effects of zooplankton grazing and the sinking rate.

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Contribution No. 323

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THE REACTION OF CERTAIN CRUSTACEA TO DIRECT AND TO DIFFUSE LIGHT

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Many plankton organisms which move downward, away from daylight in the sea, move toward a source of light in the laboratory. It was suggested in a previous paper (Schallek, 1942) that this behavior is caused by the animals' being exposed to diffuse light in nature, but to direct light in the laboratory. It was reported that when a tall glass cylinder containing the copepod *Acartia tonsa* was illuminated from above, the animals swam up toward the light. This phototropic reaction seemed to depend on the fact that the light was shining directly down the axis of the cylinder. But when the lamp was moved so as to illuminate the container obliquely, the refraction of the light at the surface of the water and its reflection from the curved inner wall of the cylinder seemed to form a diffuse illumination. Under these circumstances the animals sank downward, simulating their behavior in nature. The present paper provides a further development of this hypothesis based on measurement of the light distribution inside the cylinder.

Apparatus

A glass cylinder 18 inches high and six inches in diameter was used for the experiments. The cylinder was kept at 15° C. in a constant temperature bath 15 inches high and 15 × 18 inches in area. The bath had black walls to absorb reflected light, and was kept in a photographic darkroom. Illumination was from a 75-watt lamp with a parabolic, aluminum-coated reflector. The lamp was placed in five different positions, each 12 inches from the top of the cylinder, but separated by 22.5°. Position 1 was on a level with the top of the cylinder, while position 5 was directly overhead.

Light measurements were made with a Westinghouse "Photox" cell connected to a micro-ammeter, and calibrated in foot candles with a Macbeth illuminometer. The cell was placed in a waterproof case, and covered with a cylindrical hood, limiting the light received to an angular opening of 22.5°. The cell was held six inches below the surface of the cylinder by clamps and rods attached to a ringstand. As this apparatus would not fit inside the six-inch cylinder, an 11-inch cylinder was used for the light measurements. This change is not believed to be a significant source of error.

Twenty *Acartia tonsa* were placed in the cylinder at a time, while the lamp was shifted from position 1 (oblique) to position 5 (overhead). Alternate runs were made in the opposite direction. Two hours' exposure to each position was allowed before counting to permit the animals to reach equilibrium. Results are presented as the percentage of animals in the top third of the cylinder.

[1] Contribution No. 323.

Observations

A. tonsa moves down when the illumination is oblique, and moves up when the lamp is overhead (Table I). Measurements of the light distribution inside the cylinder are given in Figure 1.

Table I

Distribution of animals in cylinder
Average of three runs with 20 animals each

Light position	1	2	3	4	5
Elevation above horizontal	0	22.5	45	67.5	90°
	Per cent animals in top third of cylinder				
Acartia tonsa	13	29	47	65	68
Centropages typicus	69	70	72	72	79

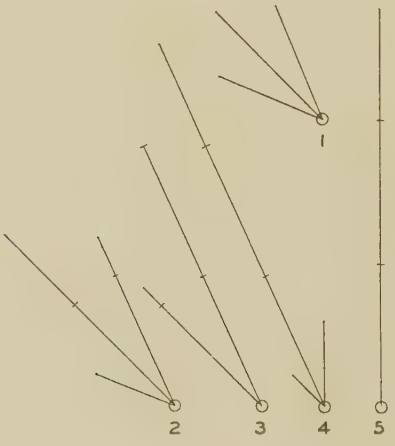

Figure 1. Light distribution inside the cylinder for each position of lamp. The small circle indicates the location of the photometer, six inches below the surface of the water. The number of each lamp position is given below the circle. The length of each line represents the light intensity recorded when the photometer was pointed in that direction. Logarithmic scale: the first crossbar = 100 foot-candles, the second = 1000 F. C. The hood limiting the incident light to 22.5° made the cell insensitive to less than 20 F. C.

These measurements reveal several factors that may influence the behavior of *A. tonsa:*

1. There is a 50-fold increase in the *intensity* of the maximum beam as the lamp is raised over the cylinder. It may be that the animals swim up in a bright light and sink down in a dim one. To test this factor, a slide-wire resistance was connected in series with the lamp, permitting the light intensity to be varied while the lamp was held in the overhead position. A 120-fold increase in the intensity of the light had no effect on the distribution of *A. tonsa* (Table IIA).

2. The *direction* of the maximum beam is vertical in position 5, but is more horizontal in the other positions. Perhaps the animals swim toward a vertical light but sink downward in a horizontal one. This possibility was tested by

TABLE II

Effect of changing light intensity

A. Cylinder vertical, light overhead.

Average of three runs with 20 *A. tonsa* each

Light intensity	10	200	1200 F. C.
Per cent animals in top third of cylinder	56	52	54

B. Horizontal trough, illuminated from end.

Light intensity		10	20	200	1300 F. C.
Time to move 10 inches					
Animal	1	130	150	125	130 seconds
	2	15	25	10	15

placing the cylinder on its side. The light from position 5 (shining directly down the axis of the cylinder) formerly was vertical, but now came in a horizontal direction. When the lamp was in position 1 (oblique) the animals sank downward (Figure 2A); when it was moved to position 5 (now horizontal) the animals swam to the end of the cylinder nearest the light (Figure 2B). Hence the animals swim toward the light when it is parallel to the axis of the cylinder, and sink downward when it is oblique, regardless of whether the light comes from a horizontal or a vertical direction.

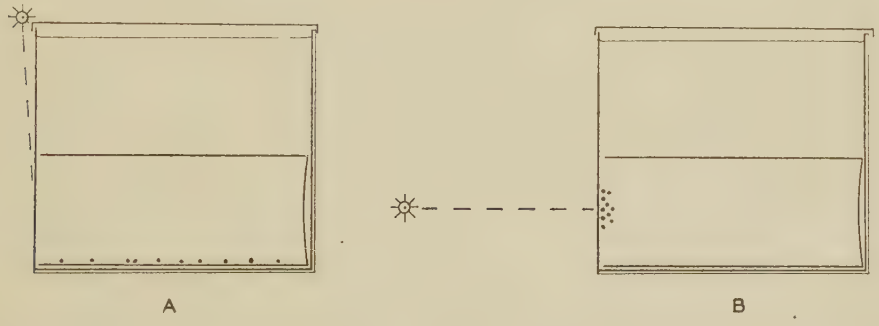

FIGURE 2. Arrangement of apparatus to test effect of light direction. A, lamp in position 1; B, lamp in position 5. Position of animals shown by black dots. The cylinder is on its side in a glass tank painted black (double line) on all sides except one through which the light enters. A piece of tarpaper covers the top.

A further test was made with a horizontal tropism trough, which could be illuminated at either end by a beam of light shining directly down the trough. *A. tonsa* always moved toward the light under these conditions; by turning on first the light at one end and then that at the other, it was possible to keep an individual moving back and forth from end to end indefinitely. Here again the reaction is independent of light intensity (Table IIB).

3. The *distribution* of the light changes as the lamp is moved. In position 5 the illumination is highly directional, with all the light coming from a single direction. In position 1 the illumination is more diffuse, the light coming almost equally from three directions. This angular distribution can be conveniently measured by the ratio (sum of intensities in other directions) : (highest intensity).

The 22.5° hood permitted 16 readings to be taken by rotating the photometer through 360°. For perfectly direct illumination, with all the light coming from one direction, this diffusion ratio will be 0 : 1, or 0. For perfectly diffuse illumination, with the light coming equally from each of the 16 directions in which measurements were made, it will be 15 : 1, or 15. The diffusion ratio for each position of the lamp is given in Table III. The curve formed by plotting these values against the percentage of animals in the top third of the cylinder is shown in Figure 3.

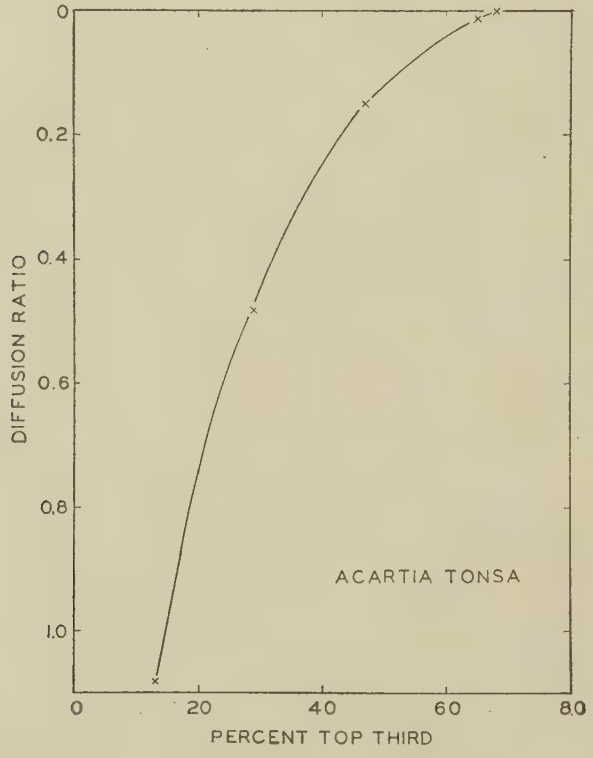

FIGURE 3. Influence of distribution of light on behavior of *A. tonsa*. Ordinate, diffusion ratio of light. Abscissa, per cent of animals in top third of cylinder.

The possibility remains that this relation is an artifact caused by the particular apparatus or procedure used. This is not so, since another copepod, *Centropages typicus*, is only slightly affected by the shift in lighting (Table I). It must therefore be concluded that the behavior of *A. tonsa* depends upon the angular distribution of the light:

1. *A. tonsa* moves toward a source of highly directional light, regardless of the intensity of the light or the direction from which it comes. This is a typical positive phototropism.

2. *A. tonsa* sinks downward in less directional (diffuse) light. This is a positive geotropism and not a negative phototropism, since the animal does not move along the beam of maximum light, but sinks passively downward.

Which of these types of illumination will animals encounter in nature? Measurements were made of light distribution in air, while the distribution of light in the sea was calculated from the data of Whitney (1941) (Figure 4, Table III).

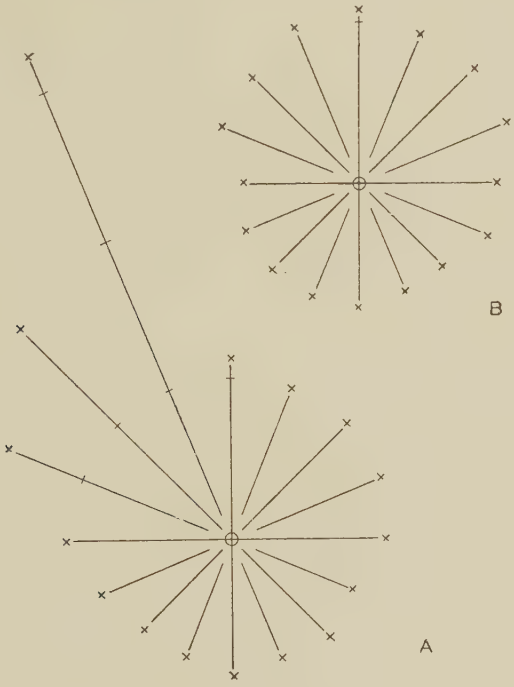

FIGURE 4. Light distribution three feet above roof of Marine Biological Laboratory, August 2, 1942, 10 A.M. Arrangement as in Figure 1.
A. Clear sky, plane of sun.
B. Clear sky, plane perpendicular to sun.

It is evident that light in the sea becomes even more diffuse than any conditions reached in the cylinder. *A. tonsa* sinks downward in diffuse light in the laboratory, and this reaction apparently accounts for the fact that it leaves the surface of the sea in the daytime (Esterly, 1928). *Centropages typicus*, which moved down only slightly in the diffuse light in the cylinder, shows only a slight downward movement in the sea (Clarke, 1934).

DISCUSSION

There are several reports in the literature of animals which react phototropically in a horizontal tube illuminated from the end, but which sink downward in a vertical tube illuminated obliquely (Table IV). Like *A. tonsa*, these animals apparently have different responses to direct and to diffuse light. Most of them show an upward movement in the dark.

The diversity of these animals, ranging from protozoans to arthropods and echinoderms, suggests that a principle of general importance is involved. A

Table III

Summary of light measurements

$$\text{Diffusion ratio} = \frac{\text{sum of other intensities}}{\text{highest intensity}}$$

Complete concentration = 0; complete diffusion = 15

		Diffusion ratio
Measurements in cylinder * six inches below surface		
Light position	1	1.08
	2	0.48
	3	0.15
	4	0.012
	5	0
Measurements in sea (Whitney, 1941)		
5 met. below surface		
Clear sky, plane of sun		2.6
perpendicular to sun		3.2
Diffuse sky, any plane		2.0
Measurements in air		
3 feet above ground		
Clear sky, plane of sun		0.13
perpendicular to sun		9.2
Diffuse sky, plane of sun		6.2
perpendicular to sun		6.8

* As the hood limiting incident light to 22.5° made the photocell insensitive to less than 20 F. C., these values are probably somewhat lower than the true ones.

Table IV

Summary of geotropism experiments

Animal	Photo-tropism in horizontal tube	Geotropism in vertical tube		Author
		In light	In dark	
Corethra plumicornis larva	+	+	−	Harper, 1907
Cyclops albidus	−	+	−	Esterly, 1907
Branchipus serratus	+	+	−	McGinnis, 1911
Daphnia pulex	+	+	−	Dice, 1914
Sagitta bipunctata	+	+	−	Esterly, 1919
Diadema setosum larva; *Paramecium*	Indif.	+	−	Fox, 1925
Holopedium gibberum	+	+	Indif.	Kikuchi, 1938
Hemimysis lamornae	+ or −	+	−	Foxon, 1940
Acartia tonsa	+	+	−	Schallek, 1942

The nauplii of *Balanus perforatus* sink to the bottom of the aquarium when taken from the dark into horizontal light, but swim up when the lamp is moved overhead (Ewald, 1912). Indif. means indifferent.

possible explanation of this effect is provided by Clark (1933). The beetle *Dineutes* moves toward the lamp if placed in a direct beam of light. If a piece of white cardboard is held perpendicular to the beam 300 cm. behind the animal, occasional circus movements appear. These become more frequent as the cardboard is brought closer to the animal, until at 10 cm. they become continuous.

This effect is attributed to the stimulation of additional ommatidia by the light reflected from the cardboard. In concentrated light, the photoreceptor will be stimulated from the front only, and the animal will then react in a typically phototropic fashion. In diffuse light, however, the photoreceptor will be stimulated from both front and side, and a different behavior appears. In the case of *A. tonsa* this results in cessation of activity, since the animal may be observed to sink passively in diffuse light.

Laboratory studies of the light reactions of animals have largely been concerned with phototropic behavior in a direct beam of light. Measurement of the light distribution in the sea shows that it is much more diffuse than in such experimental conditions. The reaction of *A. tonsa* to diffuse light in the laboratory accords with its downward movement in the ocean during the day. Its reaction in the direct light in which experiments on phototropism are usually conducted has no bearing on its behavior in nature.

Such relations may not be confined to this particular copepod. Several reports have been quoted suggesting similar behavior in other aquatic forms. The measurements of light distribution in air show that it is generally diffuse. Perhaps this will solve the riddle of why positively phototropic insects do not fly up to the sun: they may move toward a direct light but behave differently in diffuse light. Phototropism experiments in a direct beam of light need not necessarily apply to the behavior of organisms in nature.

Summary

When the copepod *Acartia tonsa* is placed in a tall glass cylinder illuminated from above, the animal swims upward. When the cylinder is illuminated obliquely, the animal sinks downward.

Measurement of the light distribution inside the cylinder shows that the behavior of *A. tonsa* depends upon the angular distribution of the light. In highly directional illumination, the animal reacts phototropically, and swims toward the light. In less directional (diffuse) illumination, the animal stops swimming and sinks passively downward.

Measurement of the light distribution in the air and in the sea shows that it is generally more diffuse than the conditions in the cylinder. The reaction of *A. tonsa* to diffuse light in the laboratory accords with its downward movement in the ocean during the day. Its reaction in the direct light in which phototropism experiments are usually performed has no bearing on its behavior in nature.

Acknowledgments

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Contribution No. 325

THE EFFECT OF COPPER UPON THE DEVELOPMENT OF BACTERIA IN SEA WATER AND THE ISOLATION OF SPECIFIC BACTERIA[1]

By

SELMAN A. WAKSMAN, DONALD B. JOHNSTONE AND CORNELIA L. CAREY[2]

INTRODUCTORY AND HISTORICAL

Copper is used extensively in the eradication of various types of disease-producing microorganisms, especially fungi, as well as other organisms which are a nuisance to water supplies, such as algae and certain animal forms. It is also known that traces of copper are essential for the growth of many of the lower forms of life. The extensive literature on the relation of copper to microbial development is largely limited to the above two phenomena. Comparatively little is known, however, of the effect of copper upon a mixed microbiological population consisting of many organisms with different metabolic processes. It is known, for example, that the growth of plants in certain soils, as peats, will respond markedly to the application of small amounts of copper (5). The development of fungi in copper-free media has been suggested as a means of determining the concentration of copper in a given soil; this method is based upon the response of *A. niger* to the presence of small amounts of available copper (3).

Certain microorganisms are known to be capable of tolerating fairly large amounts of copper. However, the organisms investigated so far are found largely among the fungi (1, 6, 7, 8). What role these organisms play in the transformation of copper in nature or even in artificial substrates is still a matter of speculation; high copper concentrations retard the growth of the organisms during the early stages of development. The presence of excessive concentrations of copper brings about the elimination of most microorganisms in a natural substrate with the exception of certain fungi capable of withstanding these high copper concentrations (6). The mycelium of some of the

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copper-resisting fungi was found (1) to contain considerable copper, which is in a fixed state and cannot be readily removed by acids. The absorption of copper during the early stages of growth was found (4) to be followed by the elimination of the copper into the medium in the older cultures.

The relationship of copper to bacteria has been chiefly limited to its disinfectant action, different bacteria varying greatly in their resistance to copper. Some of the bacteria were found (2) to take up enough copper to color them, first green, then brown.

A search for information concerning the relationships of marine bacteria to copper in sea water suggested the desirability of undertaking a study of the effect of copper upon marine bacteria as a whole and of the presence in the sea of bacteria capable of withstanding copper in concentrations greater than mere traces. Particular attention was paid to the influence of copper on the development of marine bacteria in sea water and in culture media. The problems of adjustment of bacteria to varying copper concentrations in sea water and the effect of these bacteria upon the copper have been studied.

EXPERIMENTAL

The effect of copper on marine bacteria. The medium selected for the study of the effect of copper upon marine bacteria was one extensively used in this laboratory for the study of marine bacteria and supplemented with varying amounts of $CuSO_4.5H_2O$. This medium has the following composition:

Agar	15 gm
Glucose	1 gm
Peptone	1 gm
K_2HPO_4	0.05 gm
Sea water	1000 ml

The medium was divided into several portions which received additions of $CuSO_4$ ranging from 0 to 40 mg per liter. These media were tubed, plugged, and sterilized and used for plating out fresh sea water. The numbers of bacteria found in the water taken near the dock of the Oceanographic Institution at Woods Hole showed no appreciable differences in the media receiving the above amounts of copper. Apparently these concentrations were not great enough to have an inhibiting effect upon the growth of marine bacteria. Even in this preliminary experiment, however, the appearance of several red-brown pigmented colonies on the plates containing the largest amount of $CuSO_4$ was observed.

This experiment was repeated using greater concentrations of copper in the medium, as follows:

Medium No.	$CuSO_4.5H_2O$ *mg/L*
1	0
6	50
7	100
8	200

Fresh sea water was plated out in dilutions of 1 and 1 : 10 using the above media. In addition, small amounts of fresh films from plates submerged in the water for several weeks were also plated out in dilutions of 1 : 1,000 and 1 : 10,000. The plates were incubated for two and four days at room temperature (25° C.), and the total colonies developing on the plate were determined. The red-brown colonies, which had a typical appearance of metallic copper, were designated as "copper colonies." The results obtained may be summarized as follows:

NUMBERS OF BACTERIA IN 1 ML. FRESH SEA WATER

Medium No.	*Colonies per plate, dilution* 1 : 10	
	Total colonies	*"Copper" colonies per plate*
1	200	0
6	160	10
7	3	0
8	0	0

NUMBERS OF BACTERIA IN A SMALL AMOUNT OF FILM FROM SUBMERGED SURFACE

Medium No.	*Colonies per plate,* 1 : 1,000 *dilution*	
	Total colonies	*"Copper" colonies*
1	>500	0
6	>500	a few
7	500	many
8	90	0

On continued incubation of the plates, the red color of the "copper" colonies on the media containing $CuSO_4$ disappeared, the colonies losing their red pigment and becoming white again.

There was a slight increase in acidity of the medium with an increase in copper concentration; however, even the highest concentration of copper used in this experiment did not make the medium very acid, changing the pH to 6.9. It is believed that these copper concentrations would not have had a great enough effect upon the growth of the bacteria merely through the pH change.

It was apparent from the results of these experiments that the presence of $CuSO_4$ in the medium was responsible for the pigmented colonies, and the presence of 200 mg of $CuSO_4.5H_2O$ per liter of

medium was too great a concentration for the development of the total bacterial population in the water, as well as of the specific "copper" present. The addition of 100 mg $CuSO_4.5H_2O$ per liter of medium was found to be the optimum for the development of the specific "copper" colonies on the plate. This concentration was used in the subsequent experiments for determining the abundance of the "copper" organisms in sea water. The medium containing this amount of copper will henceforth be referred to as medium 7.

In order to determine whether such "copper" bacteria are also present in soil and in lake water, samples of ordinary soil and pond water were plated out, using nutrient agar media enriched with 100 mg of $CuSO_4.5H_2O$ per liter. The materials were plated out using different dilutions. The numbers of bacteria obtained on this medium were 1,600 per ml of pond water and 60,000 per gm of soil, after 2 days incubation. No typical "copper" colonies appeared on any of the plates. Whether these bacteria are totally absent in soil and in fresh water or whether the salts present in the media used for plating out the sea water had a specific effect upon the development of such bacteria, still remains to be determined.

The effect of additions of various forms of copper to sea water upon the general bacterial population of the water as a whole and upon the development of specific "copper" bacteria was now studied.

The influence of $CuSO_4$ *upon the bacteria and upon oxygen consumption in sea water.* The total numbers of bacteria in the water were measured by the plating method, using the copper-free medium 1; the number of "copper" bacteria were determined by the use of medium No. 7. Oxygen consumption in the water, as influenced by the addition of varying amounts of copper, was also measured by the common Winkler method.

Several preliminary experiments were carried out for the purpose of determining the type of container to be used for incubating the water, the method for treating the water, and the length of the incubation period (Table I).

Only the results of one typical preliminary experiment are reported here. Measured quantities of fresh sea water received varying amounts of $CuSO_4.5H_2O$. They were immediately distributed in oxygen bottles (230 ml portions), which were incubated under water at room temperature (20°–25° C). After different periods of incubation, the bottles were removed and contents used for plating and oxygen determination. One ml portions were removed with sterile pipettes, disturbing the water in the bottles as little as possible. The

TABLE I—Influence of Copper upon the Development of Bacteria and Consumption of Oxygen in Fresh Sea Water Kept in Laboratory

Incubation of water, days	*Copper added as* $CuSO_4.5H_2O$ *mg/l.*	*Oxygen consumed ml/l.*	*Total numbers of bacteria per 1 ml water*	*"Copper" bacteria per 1 ml water*
0	0	0	2.000	.45
1	0	1.204	3.160.000	232.000
1	1	0.777	1.660.000	720.000
1	10	0.018	38.000	6.500
1	50	0.021	73.500	5.000
1	200	0.070	105.000	17.000
2	0	1.708	2.400.000	35.000
2	1	1.544	4.400.000	166.000
2	10	0.679	1.050.000	163.000
2	50	0.665	1.550.000	115.000
2	200	0	24.000	1.500
4	0	2.216	220.000	1.000
4	1	2.590	3.440.000	21.500
4	10	0.952	3.760.000	42.500
4	50	0.892	6.540.000	290.000
4	200	0.574	703.000	682.000
7	0	2.976	200.000	2.000
7	1	4.480	22.000	1.500
7	10	0.952	2.510.000	1.050.000
7	50	0.795	650.000	360.000
7	200	0.795	810.000	31.500

reagents for the oxygen determination were then added to the residual water in the bottles. The bacterial plates were incubated at room temperature for 4 days and counted at the end of the 2d and 4th days. The 4 day counts are reported unless otherwise indicated.

Although the results of this experiment are somewhat erratic, nevertheless they permit certain interesting conclusions. The addition of copper to the water had at first a depressing effect upon bacterial development and oxygen consumption. This effect was soon overcome; the smaller the amount of copper added the shorter was the period of "depression" or "adaptation." Subsequently copper had a stimulating effect, depending on the amounts of copper added; the less the concentration of copper the sooner the stimulating effect was observed. At the highest concentration of copper (200 mg $CuSO_4.5H_2O$), the period of depression was longest and was not completely overcome even after 7 days incubation, the longest period used in this experiment.

In order to overcome the variability in the results which definitely appeared to be due to experimental errors, this experiment was repeated, using a larger number of replicates and extending the incubation period for a somewhat longer time. Fresh sea water, taken some 100 feet away from the pier of the Oceanographic Insti-

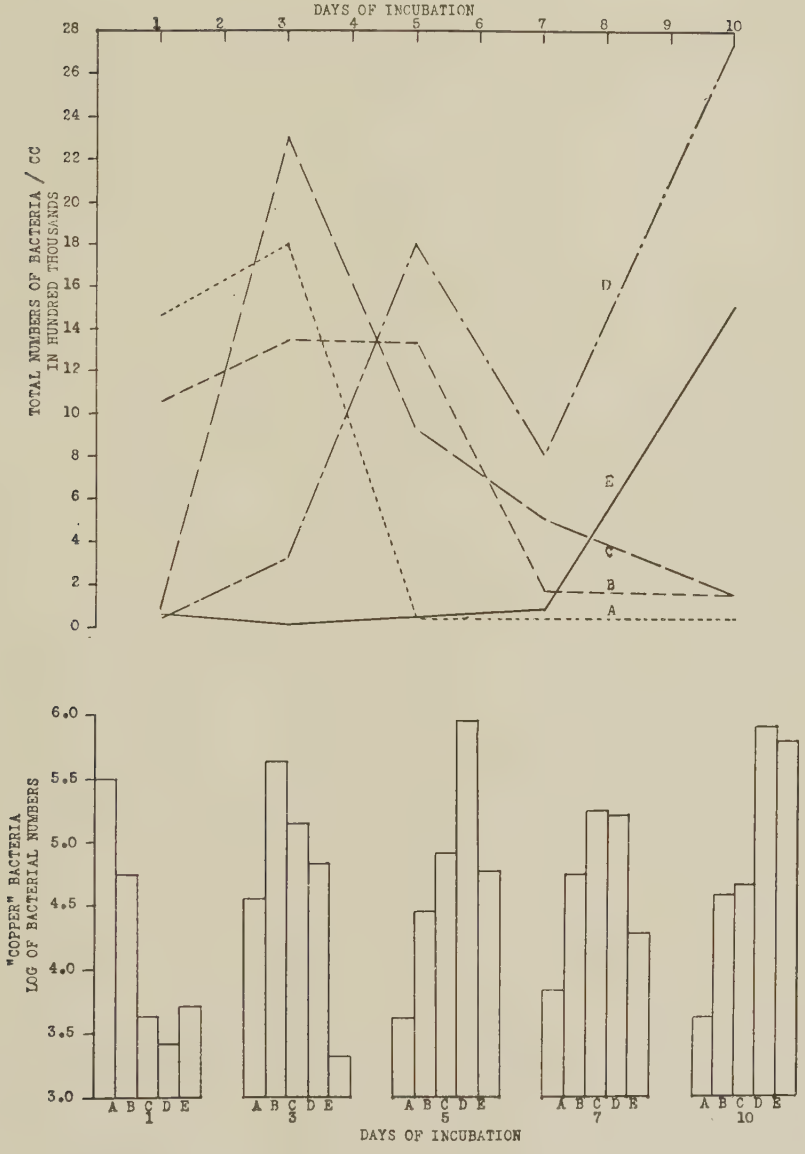

Figure 38. Influence of copper sulfate upon the total bacterial population of sea water and upon the development of "copper" bacteria. $CuSO_4.5H_2O$/L Sea water: (A.) none; (B.) 1 mg; (C.) 10 mg; (D.) 100 mg; (E.) 500 mg.

tution, was placed in fifty oxygen bottles, each containing about 150 ml water. These bottles were divided into 5 groups of 10 each. Group A received no copper, B—1 mg $CuSO_4.5H_2O$ per liter of fresh water, C—10 mg, D—100 mg, and E—500 mg $CuSO_4.5H_2O$. The

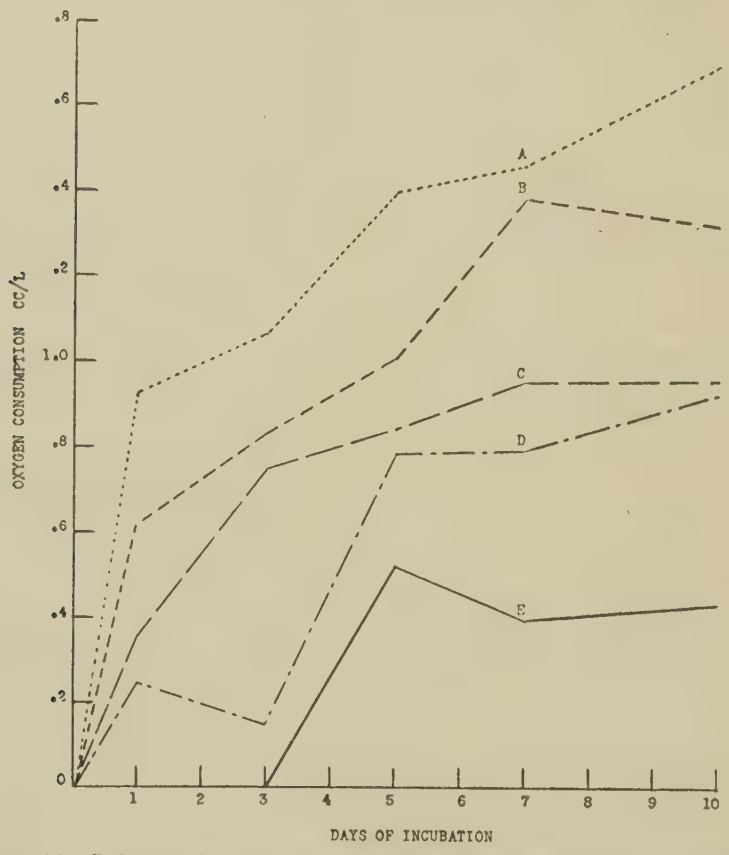

Figure 39. Influence of copper concentration upon the consumption of oxygen in sea water. $CuSO_4.5H_2O$/L: (A.) none; (B.) 1 mg; (C.) 10 mg. (D.) 100 mg; (E.) 500 mg.

bottles were incubated under water. Two bottles from each lot were removed for analysis after 1, 3, 5, 7, and 10 days.

The original fresh sea water contained 670 bacteria and 90 "copper" bacteria per 1 ml of water. The oxygen content of the water was 5.09 cc/liter. Figures 38 and 39 present graphically the results of this experiment. Here again, the addition of copper to the water had a delaying effect upon bacterial development, which was later overcome;

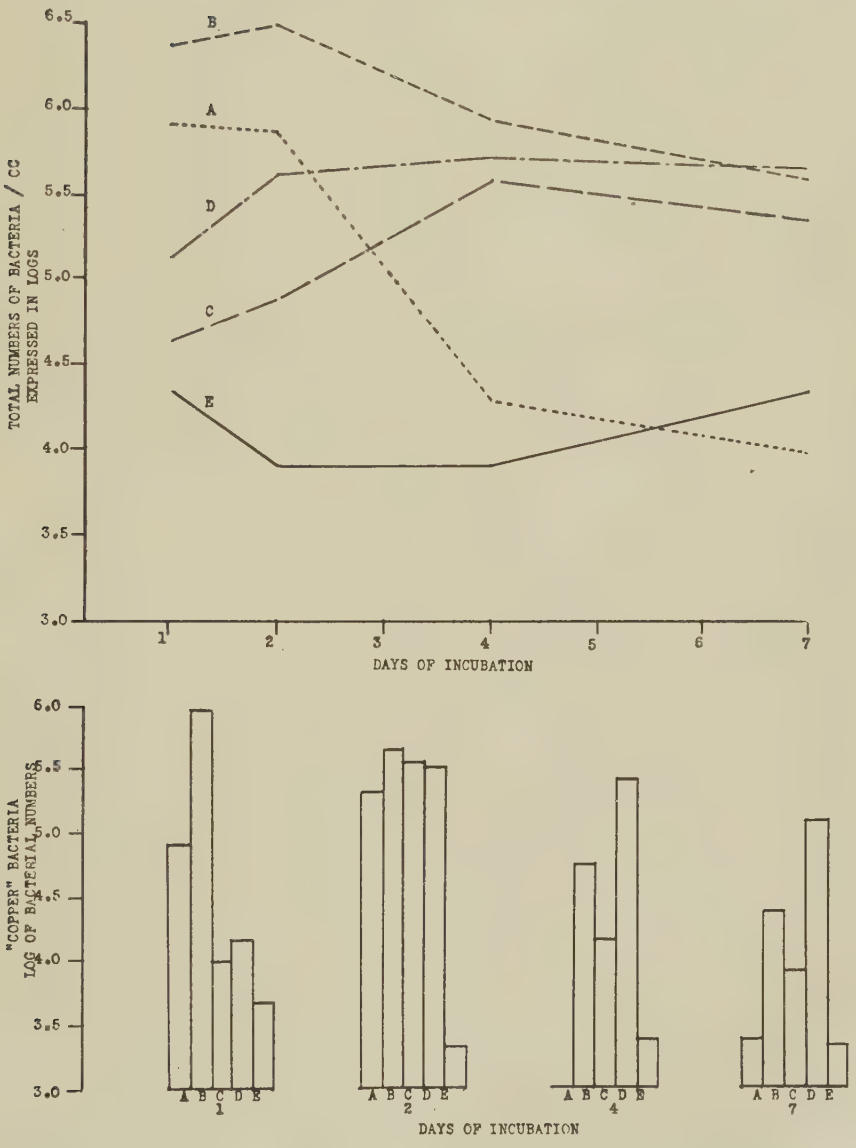

Figure 40. Influence of copper chloride upon the total bacterial population of sea water and upon the development of "copper" bacteria. $CuCl_2$/L; (A.) none; (B.) 1 mg; (C.) 10 mg; (D.) 50 mg; (E.) 100 mg.

the larger the amount of copper added, the greater was the length of the depression period. No explanation can be offered for the fact that curve B, for the water receiving the smallest amount of copper, did not show the stimulating effect obtained in the previous experiment; this may possibly be due to the omission of the two day incubation period, when such a rise might have been observed. The effect upon the consumption of oxygen in the water was also not stimulating but depressive, this effect increasing with an increase in the amount of copper added.

The influence of $CuCl_2$ *upon bacterial development and upon oxygen consumption in sea water.* In order to avoid the secondary effect of

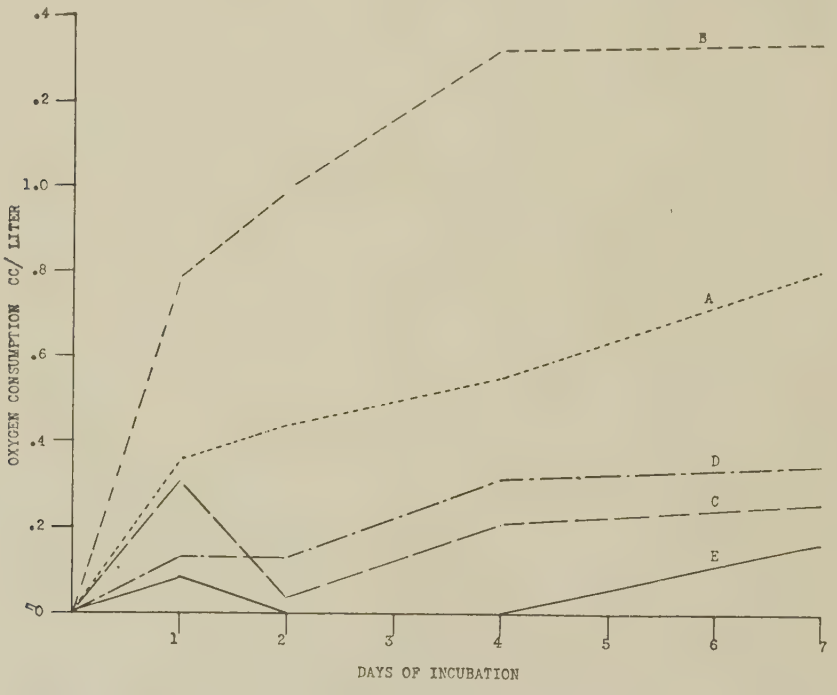

Figure 41. Influence of copper chloride upon the oxygen consumption by bacteria in sea water. $CuCl_2$/L: (A.) none; (B.) 1 mg; (C.) 10 mg; (D.) 50 mg; (E.) 100 mg.

the sulphate ion upon bacterial development, copper chloride was now employed. This experiment was carried out in a manner similar to the previous one. Five series of bottles were used. These received fresh sea water containing the following amounts of $CuCl_2.2H_2O$ per liter of water.

Series	$CuCl_2.2H_2O$ mg/L
A	0
B	1
C	10
D	50
E	200

The bottles were incubated as in the previous experiments and the bacterial numbers and oxygen consumption were determined. The bacteria were plated out on media 1 and 7, in dilutions of 1 : 1,000 and 1 : 10,000. The fresh sea water, plated out at the beginning of the experiment, gave a total of 16,960 bacteria per 1 cc of water using medium 1 and 1,840 "copper" bacteria on medium 7.

The results of this experiment (Figures 40 and 41) are very similar to those obtained with $CuSO_4$. Copper in the form of $CuCl_2$ had, in small quantities, a stimulating effect upon the growth rate of marine bacteria and especially upon oxygen consumption in the water. One mg. of $CuCl_2.2H_2O$ per liter of sea water gave a marked increase in the total numbers of bacteria as well as in the numbers of "copper" bacteria. The addition of 10 mg or more of the copper salt per liter of water showed, however, marked inhibiting effects, which tended to be overcome on further incubation. The largest amount of copper salt added had the greatest depressive effect; the period of depression was practically ended after 7 days' incubation of the water in the laboratory.

The influence of Cu_2O *on bacterial development and oxygen consumption in sea water.* A third form of copper, Cu_2O, was now used for determining the effect upon bacterial activities in sea water. This experiment was carried out in a manner similar to the previous ones. An incubation period of 14 days was used.

The water was treated as follows:

Series	Cu_2O mg/L
A	0
B	1
C	5
D	20
E	100

The fresh sea water plated out at the beginning of the experiment gave 238 bacteria on medium 1 and 10 "copper" bacteria on medium 7 per 1 ml of water. The oxygen content of fresh water was 4.99 cc/liter. This is similar to that found in the other experiments; however, when the water was tested immediately after the Cu_2O was

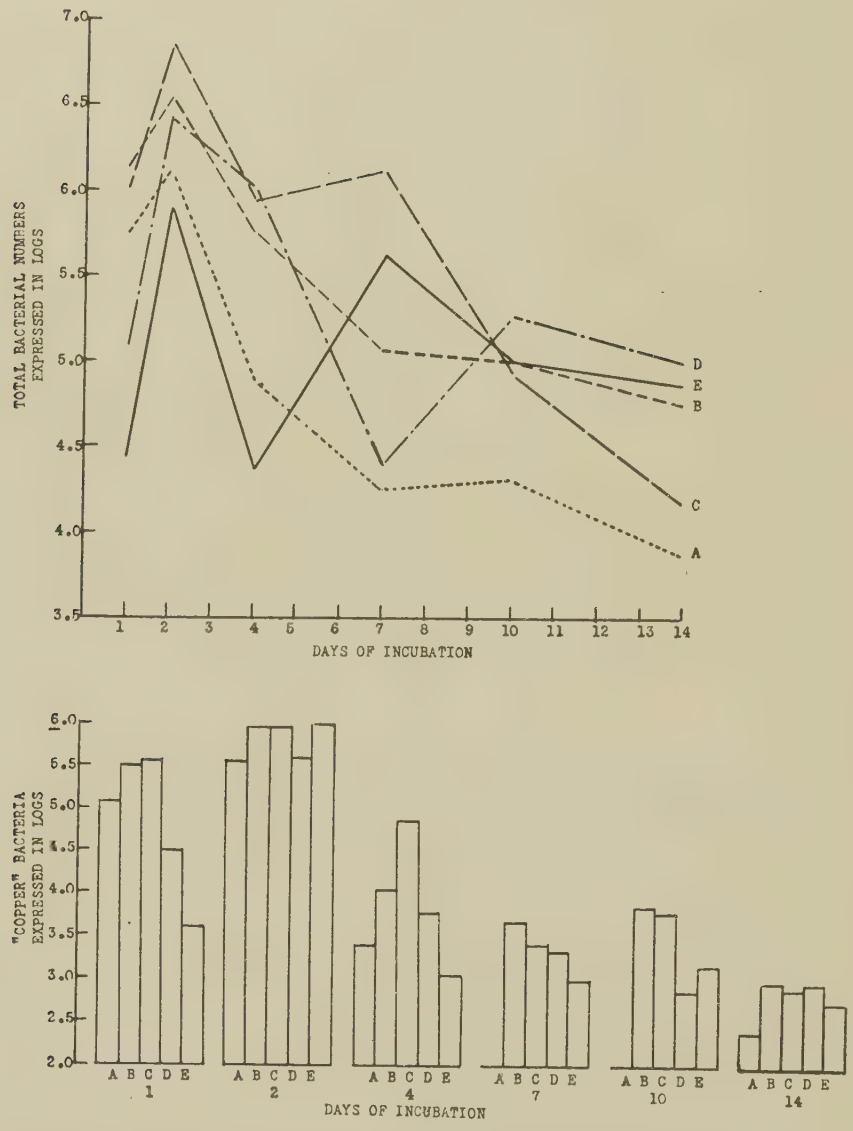

Figure 42. Influence of copper oxide upon the total bacterial population in sea water and the development of "copper" bacteria. Cu_2O/L sea water. (A.) none; (B.) 1 mg· (C.) 5 mg; (D.) 20 mg; (E.) 100 mg.

introduced, there was an immediate marked consumption of the oxygen. This was due to the oxidation of the cuprous oxide to cupric oxide. This reaction consumed approximately 1.5 cc of oxygen per 1 liter of sea water in the E series, to which the highest amount of Cu_2O was added.

This experiment was repeated in order to determine the amount of oxygen taken up chemically and biologically by the addition of Cu_2O to the fresh water. Two lots of bottles were filled with fresh sea water, one lot receiving no copper and the other receiving 100 mg of Cu_2O per liter of water. These bottles were incubated for short period of time and analyzed for oxygen. The results reported in Table II definitely point to the fact that the chemical consumption of the oxygen is immediate and does not increase on further incubation.

The results of the effect of Cu_2O on bacterial development presented in Figure 42 confirm fully the effects of the other two forms of copper, namely, at first a depression followed by a stimulating effect. The greater the amount of copper added, the longer was the depressive action. There were, however, two exceptions; first, no depressive action was observed where the two smallest amounts of copper were added as Cu_2O; second, the depressive effect of the largest amount of copper was attained with the 7 day incubation period.

TABLE II—Chemical and Biological Oxygen Consumption in Water Treated with Cu_2O

Incubation of water, hours	*Water enriched with* Cu_2O	*Unenriched water*	*Biological Consumption*	*Chemical Consumption*
Start	3.41	5.00	0	1.59
2	3.32	4.98	0.02	1.66
4	3.21	4.93	0.07	1.72
6	3.16	4.93	0.07	1.77
8	3.16	4.88	0.12	1.72
10	3.16	4.73	0.27	1.57
24	3.16	4.34	0.66	1.18 (?)
72	2.28	3.82	1.18	1.54

100 mg Cu_2O per liter of water. Data expressed in cc of oxygen per liter of water

"Copper" bacteria in surface films and copper concentration by "copper" bacteria. A series of films removed from various surfaces which had been submerged near or at the dock of the Oceanographic Institution were examined for the total bacterial content and the percentage of "copper" bacteria. Uniform pieces of film were removed, by means of sterile swabs, suspended in sterile sea water, diluted and plated. The results (Table III) showed no particular uniformity between the total numbers and the numbers of "copper" bacteria. This may have been due to the fact that the films were of

different ages (42–170 days old) and varied considerably in thickness; the amounts of film removed from the different surfaces were also not absolutely uniform. The "copper" nature of some of the bacteria was not always recognized, since the pigmentation of the colonies and the disappearance of the pigment did not take place uniformly.

The results of these experiments point definitely to the fact that copper, in various forms, has some very definite relations to the development of bacteria in sea water. First, there is an apparent adaptation of the bacteria to the copper, whereby the latter may even exert a stimulating effect upon the development and activities of the marine bacteria, within definite limits of concentration and time. Second, there is a marked development, among the marine bacteria, of a specific population, which has been designated as "copper" bacteria; these are present in the water and are capable of developing there in very large numbers.

In order to demonstrate whether the copper is being concentrated in the bacterial pellicle, an attempt was made to produce such pellicles artificially. Sea water was enriched with the nutrients of medium 7 and placed in shallow layers in flasks. Some of these were left in an unsterile condition and others were sterilized and inoculated with some of the pure cultures of the "copper" bacteria. Typically red pellicles were produced in some of the flasks. These pellicles were carefully removed and analyzed for copper. It was found that about 8 per cent of the copper added to the medium was concentrated in the pellicles. Only a few analyses have been made so far, however, and further work is required in order to establish this point. Because of the great variability in the pigmentation of the pellicle and the limited analyses made so far, these results should be taken as merely suggestive. The oxygen tension of the medium appears to play an important role in this connection.

TABLE III—Numbers of Total Bacteria, Developing on Plate, and of "Copper" Bacteria in Comparable Units of Bacterial Films from Different Surfaces

	Medium 1 (no copper)	Medium 7 (copper present)	
No. of submerged plate	*Total numbers of bacteria*[1]	*Total numbers of bacteria*	*"Copper" bacteria*
0	428.000	57.000	8.000
10	1.295.000	361.500	28.500
14	1.680.000	124.500	20.500
20	1.095.000	102.500	7.000
25	1.150.000	114.500	12.000
28	930.000	103.500	21.500
33	400.000	107.000	4.000

[1] Per unit of surface removed by sterile swab.

Nature of the "copper" bacteria. A detailed study of the nature and physiology of the "copper" bacteria will be presented at a later date. It is sufficient to present here only some of their general characteristics in order to emphasize their specific nature.

The development of the "copper" colonies on the agar plate containing a copper salt was rather characteristic. The surface colonies behaved in this respect quite differently from the sub-surface colonies. The former produced round reddish to red-brown and even dark-red colonies; the pigment of the colonies gradually disappeared, either beginning from the center or from the periphery of the colony, until, after 5–6 days, the whole colony lost its red pigment. The sub-surface colonies kept the pigment for a longer period of time. This phenomenon did not appear to be correlated with sunlight. Whether the copper is at first assimilated or absorbed by the cell, then oxidized to give the pigmented form, and finally reduced or excreted remains to be determined. It was observed, however, that when a quantity of bacterial cell material was collected, dried and analyzed, it rapidly lost its red color and became greenish.

In order to study further the physiological characteristics, these bacteria were isolated in pure culture and cultivated on a special copper-containing medium. This medium was comparable to soft agar, thus enabling the organism to grow under partial anaerobic conditions. An extract of *Chondrus crispus* (Carragar) was used as the gel-forming agent. This medium, designated as 6b, had the following composition:

Peptone	1 gram
Glucose	1 gram
K_2HPO_4	0.05 gram
$CuSO_4.5H_2O$	0.1 gram
Carragar	6 grams
Sea water	1,000 ml.

This medium was placed in test tubes and sterilized. When the medium was allowed to set, it separated into two distinct layers; the lower layer was of a gel nature, whereas the top layer was in the form of a supernatant fluid. This enabled the bacteria to grow on the surface of the liquid; when a pellicle of sufficient thickness was produced, it dropped upon the lower or gel layer. The pellicle thus accumulated, without rapidly losing its red color, until several layers of film were formed, one on top of the other.

Colonies were picked from the various plates containing media 1 and 7 and inoculated upon this special medium. Both white and red colonies were picked from the copper-containing medium 7 and white colonies alone were picked from the copper-free medium 1.

These pure cultures were incubated in the dark at room temperature. The red color of the pellicle served as evidence of the "copper" character of the bacteria. Some cultures produced this coloration faster than others. Those colonies which were red on medium 7 gave red growth on medium 6b and those colonies that were white on medium 7, remained white on the 6b medium, whereas the white colonies picked from medium 1 gave either white or red growth on medium 6b. This indicates that the "copper" colonies are masked on medium 1 by the absence of copper in the medium; it tends to prove further that the "copper" bacteria are able to live in the absence of copper in the medium.

The cultures first isolated from colonies were replated, reisolated several times, and retransferred to media 6b and 7. Each reacted true to type. They were separated on the basis of pigmentation characteristics, size and appearance of colony. Thirteen pure cultures of "copper" bacteria were thus selected for further study.

On microscopic examination, various cultures of "copper" bacteria were found to be very small, short rods. Those taken from the pigmented colonies appeared darker and were more readily recognized than the bacteria taken from the white colonies, which had either lost their red-brown pigment or had not acquired it yet. They were found to behave alike toward the gram stains and were gram-negative organisms without any spores.

When plated out, the "copper" bacteria produced colonies both on the surface and below the surface of the agar. The surface colonies pigmented very rapidly, usually within 48 hours, and remained pigmented from two to four days. They were convex, had a very smooth edge and the surface was smooth and shiny. In losing the pigment, the colonies did so in a very characteristic manner. The pigment was gained or lost in the form of concentric zones until finally the colony no longer possessed any pigment at all. This occurred very rapidly in the surface colonies, and often only one concentric ring was noted. In the deep colonies, however, the rate of pigmentation was slower, usually requiring from four to five days to appear; the pigment remained from four to ten days depending on the number of colonies in close proximity to one another. The deeper colonies continued to produce concentric rings of red and white to the extent of seven or eight in number. The areas of white between the rings of red retained a more or less granular appearance. The reason for the occurrence of the rings is not definitely known, but it is thought that the copper nearest the youngest cells, the periphery of the colony, is responsible for the pigmentation, and, as the colony enlarges, this area is no longer adjacent to the fresh supply of copper, and becomes white again.

In the case of streaks of the bacterial cultures, grown on medium No. 7, the pigmentation occurred at the base, the culture continuing to grow along the entire streak. The growth was usually shiny and echinulate in appearance. In time, it became tough and leathery, as did the colonies as well. The culture remained alive but it could be transferred only by removing the whole colony or a mass of growth from the agar. The cultures of the "copper" bacteria died in a relatively short time under aerobic conditions, but not under reduced oxygen pressure. For this reason, the pure cultures are kept under partial anaerobic conditions. This can be accomplished by using deep tubes of soft agar or carragar medium.

CONCLUSIONS

1. The presence of 100 mg $CuSO_4.5H_2O$ in a sea water medium allowed the development of certain red-brown pigmented colonies, from sea water and from bacterial films taken from submerged surfaces. No such colonies were obtained from fresh water or from soil by the use of fresh water media containing the same copper salt.

2. The addition of various forms of copper to sea water had at first a depressing effect upon bacterial development and oxygen consumption. This effect was later overcome, and with time a stimulation resulted. The rapidity of adjustment of the bacteria to the addition of copper depended upon the amounts of copper added to the water. High concentrations often proved toxic to a point where the majority of the bacteria were unable to become adjusted, at least within the short experimental period employed.

3. The consumption of oxygen in sea water by bacteria was parallel to the growth rate of the bacteria, as influenced by the amounts of copper added to the water.

4. Sea water and surface films were found to contain bacteria that have a definite affinity for copper, since they are able to withstand concentrations of 500 mg $CuSO_4.5H_2O$ per liter of medium.

5. When grown upon media containing copper, the "copper" bacteria produce colonies pigmented a red-brown color, whereas no color is noted when the same bacteria are grown in the absence of copper. These "copper" bacteria were also grown in the presence of various forms of copper, including $CuSO_4.5H_2O$, $CuCl_2.H_2O$ and Cu_2O.

6. The pigmentation of the surface "copper" colonies was observed on the plates within 48 hours incubation; it persisted for two to four days. The pigmentation of submerged colonies appeared within four or five days' incubation of the plates and lasted from four to ten days.

7. A number of cultures designated as "copper" bacteria and producing red pigmented growth on media containing copper in the form of $CuSO_4.5H_2O$ were isolated. These bacteria were all small, non-spore forming, gram-negative rods.

8. The red-brown pigment in the sub-surface colonies persisted for a long time; however, in surface colonies it rapidly disappeared, in a characteristic manner, namely through the formation of concentric colorless zones which gradually turned the whole colony colorless.

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THE HISTOGENESIS AND CYCLIC PHENOMENA OF THE SINUS GLAND AND X-ORGAN IN CRUSTACEA

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Introduction

In the decade that has followed Hanström's (1933, 1934) description of the sinus gland and X-organ in Crustacea a number of investigators, Sjögren (1934), Hanström (1937), and Ståhl (1938) have described them in detail. All of these studies have been concerned with a description of these glands as they appear in the adult animal. Since there has been little or no work done upon the histogenesis of either the sinus gland or X-organ, it is one of the objects of this paper to describe the histogenesis of both the sinus gland and X-organ in detail.

The endocrine activity of the sinus gland has been more or less well established through numerous studies in the past several years. As these are quite adequately and critically examined by Scharrer (1941) and Kleinholz (1942) there is no need to review the literature in detail. For further information of this nature one should examine those papers. Although extensive physiological studies have been made in relation to the endocrine function of the sinus gland, there have been no cytological studies made (except in Cambarus by Dethier 1942) to determine whether or not there are any evidences of cyclic phenomena in this gland. The role of the X-organ has been suggested, but no cytological studies have been made of it. Both the X-organ and sinus gland have been cytologically examined and the results of this study are reported herein.

Methods and Materials

The histogenesis of the sinus gland and X-organ were studied in two species of Crustacea, *Homarus americanus* and *Pinnotheres maculatus*. The adults of these species and of *Cambarus virilis* were studied for cytological evidences of cyclic phenomena during the moulting period.

The eggs of Homarus were fixed in Carnoy-Lebrun: the first four stages after hatching were fixed in Zenker-formol and Bouin-Duboscq-Brasil, and the adult eye stalks (one week, 48 hours, six hours before, during, six hours, 48 hours, one,

[1] Contribution No. 326 from Woods Hole Oceanographic Institution.

one and one-half, four, six and thirteen months after moulting) [2] were fixed in Zenker, Zenker-formol and Bouin-Duboscq-Brasil. The eggs and first four stages after hatching were doubly imbedded in parlodion and paraffin and sectioned at five to nine micra. In some of the adults the exoskeleton of the eye stalk was decalcified and the whole eye stalk was doubly imbedded in parlodion and paraffin and sectioned at seven to 12 micra. In other adults the exoskeleton of the eye stalk was removed and the specimens were singly imbedded in paraffin. These were sectioned at seven to 15 micra.

The eggs, first zoea and adult stages (before, during, after and between moulting periods) of Pinnotheres were studied. The eggs and first zoea were fixed in Carnoy-Lebrun, Zenker-formol and Bouin-Duboscq-Brasil, and were doubly imbedded in parlodion and paraffin. Sections were cut at four to seven micra. The various stages of the adult were fixed in Zenker-formol and Bouin-Duboscq-Brasil, and were doubly and singly imbedded and sectioned at six to 12 micra.

The eye stalks of Cambarus were treated in the same way as those of Homarus; some were singly and some doubly imbedded. Sections were cut at seven and nine micra.

Serial sections were made of all specimens and these were stained with haemalum and eosin, Mallory's triple, Foot's (1933) and Lillie's modifications of the Masson trichrome stain, and the protargol method of Bodian (1937).

Observations

A. *Pinnotheres maculatus*

The X-organ is found in the embryo just before hatching (Figs. 1, 19) in that part of the eye which will become the median ventral side of the eye stalk in the

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Plate I [3]

The histogenesis of the sinus gland and the X-organ in *Pinnotheres maculatus*. All figures are oblique frontal sections of the right eye stalk. The neuropile of the optic ganglion is white and the ganglion cell layer is stippled.

Figure 1. Section of the late egg stage showing the position of the X-organ in relation to the structures of the optic ganglion. X—X-organ. S. G.—Sinus gland.

Figure 2. Section of the first adult stage showing the positions of the sinus gland and X-organ: both are distal to the medulla terminalis.

Figure 3. Section of the second adult stage. The sinus gland has begun to move distally, but the X-organ is found in the same general position as in earlier stages.

Figure 4. Section of the third adult stage. The sinus gland has advanced to a point between the medulla interna and medulla externa.

Figure 5. Section of the fourth adult stage. The sinus gland is lateral to the medulla externa at this stage.

Figure 6. Section of the fifth adult stage. The sinus gland now occupies a position between the medulla externa and lamina ganglionaris.

Figure 7. Section of the sixth adult stage. The sinus gland has advanced to a point that is distal to the lamina ganglionaris. In this as in previous stages the X-organ is found distal to the medulla terminalis.

[2] I am indebted to Dr. Charles J. Fish and the staff at the Wickford Hatcheries for the determination of the times in those specimens that were about to moult.

[3] All figures have been drawn with the aid of a micro-projection apparatus. All structures found between the hypodermis of the exoskeleton and the optic ganglia have been omitted for the sake of clarity.

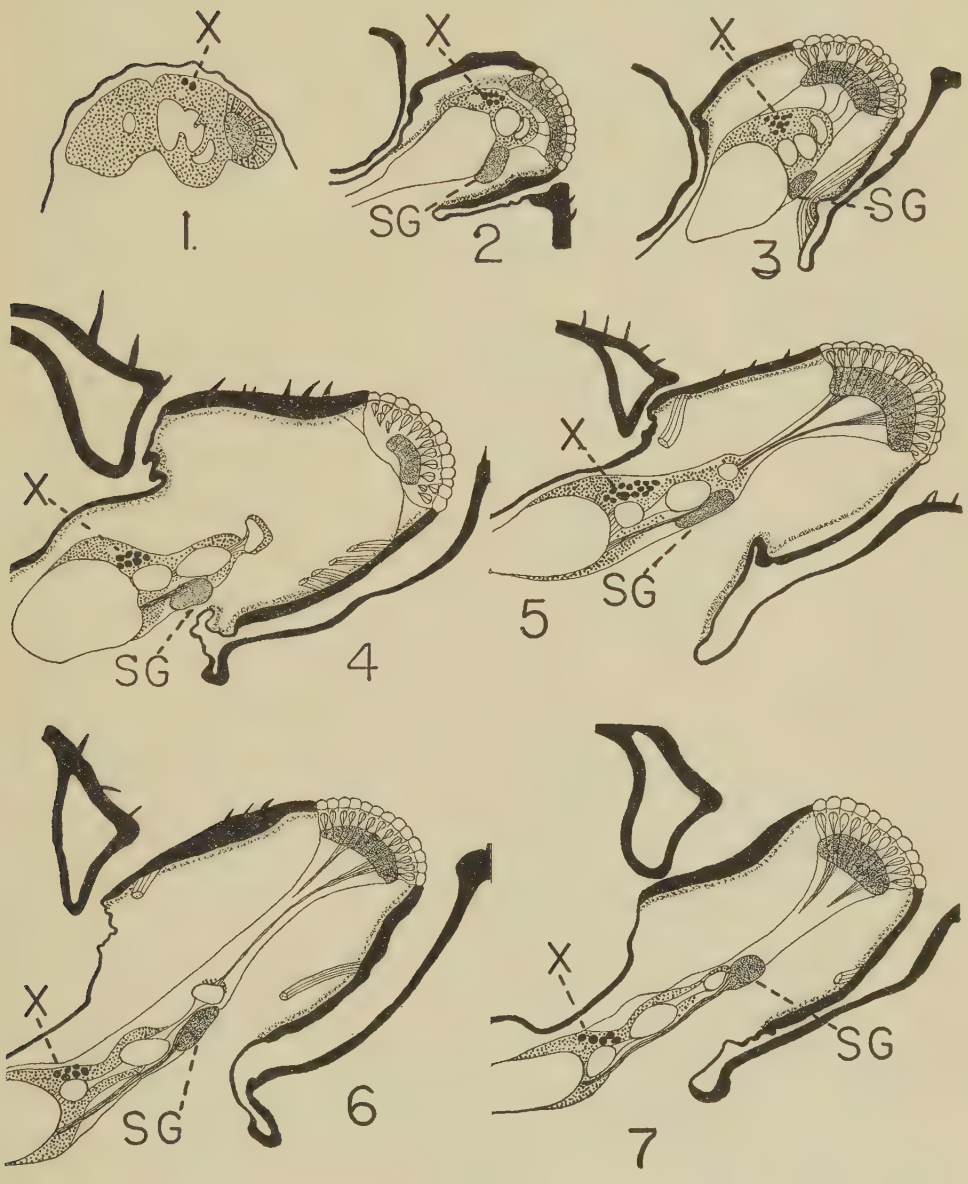


PLATE I

first zoea. It occupies a position on the distal portion of the medulla terminalis and is almost entirely surrounded by the cells of the optic ganglia. There is also found, usually between the X-organ and the medulla terminalis, an area which is devoid of cells; this appears in serial sections as a hole. As far as can be determined from cytological preparations the cells of the X-organ are very similar to those found in the ganglion cell layer. They are probably derived from the same embryonic source and later become differentiated into X-organ cells. There is no evidence of any nerve fiber connections with the medulla terminalis. The nuclei are of the same size, shape and appearance as the nuclei of the ganglia cells. There is more cytoplasm present than in the ganglia cells; it is non-granular and clear. The X-organ is an integral part of the ganglia cell layer and is not set apart from it by a connective tissue sheath.

The secretory products of the X-organ are large rounded masses which exhibit concentric rings; this seems to indicate that the secretions have been laid down at different intervals. These secretory products always give a basophilic reaction when stained; they are blue after aniline blue and are structurally very similar to those found in the X-organ of Homarus. There is no evidence of any cyclic phenomena in the egg stage as the secretions have the same characteristic appearance in all specimens.

There is no evidence that the sinus gland has been developed by the late egg stage or the first zoeal stage. Unfortunately, conditions existing at Woods Hole last summer did not permit obtaining the intermediate forms between the first zoeal and the first adult stage so that these could not be studied. Attempts to raise them beyond the first zoeal stage were fruitless.

The adults may be grouped into six categories or stages which correspond to the moults. This is comparable to the five moults found in *Pinnotheres pisum* by Atkins (1926). In all stages of the adult (Figs. 2–7, 21) the X-organ is found in the same relative position that it occupies in the egg stage. The number of cells of which it is composed increases after each moult, but no mitoses were observed at any time. The cells are larger than the ganglia cells which surround them. They are wedge-shaped and are grouped in such a way as to remind one (when examining serial sections) of a pie that has been cut; the nuclei are found around the periphery and each cell becomes narrower as its cytoplasm extends toward the center of the X-organ. As the cytoplasm becomes filled with the secretory products the nucleus is pushed more and more toward the periphery

PLATE II [4]

The histogenesis of the sinus gland and X-organ in *Homarus americanus*. All figures are oblique frontal sections; figures 8, 9, and 11 are of the left eye stalk, and figure 10 is of the right eye stalk. The neuropile of the optic ganglion is white and the ganglion cell layer is stippled.

FIGURE 8. Section of the late egg stage showing the position of the X-organ.

FIGURE 9. Section of the third stage after hatching. The sinus gland is seen as a thin structure on the proximal side of the medulla externa.

FIGURE 10. Section of the fourth stage after hatching. The sinus gland still occupies a position on the proximal side of the medulla externa. The X-organ extends to the hypodermis of the exoskeleton where the eye papilla is found.

FIGURE 11. Section of the adult stage. Both the X-organ and sinus gland, on opposite sides of the eye stalk, are seen extending beyond the limits of the neurilemma which surrounds the optic ganglion.

[4] See footnote 3.

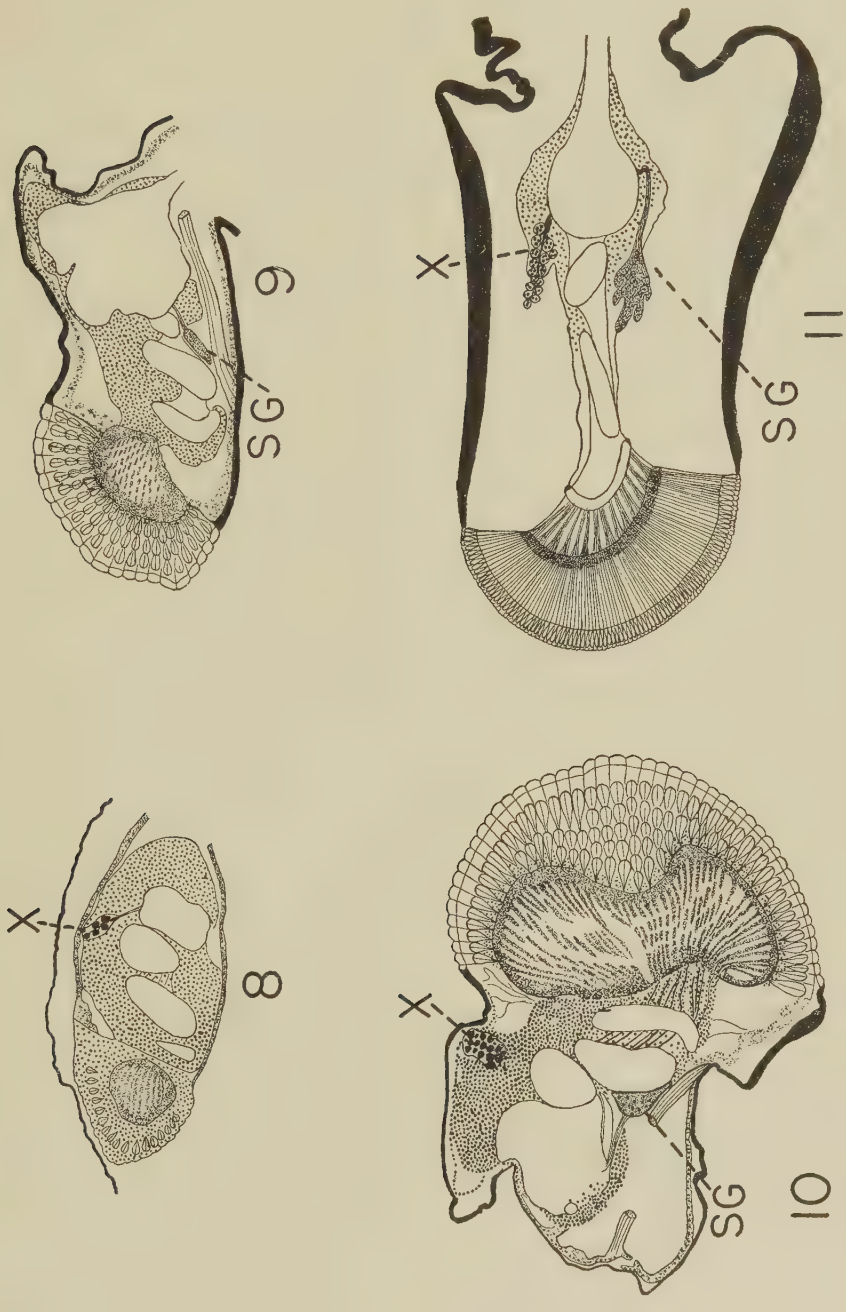


PLATE II

of the cell. In no stage is there any evidence that the X-organ has a nerve fiber tract which extends from it to the medulla terminalis. Cytologically there is no evidence of any cyclic phenomena associated with the secretion processes of the X-organ at any time. It always exhibits the same basophilic reaction regardless of whether it is fixed before, during, after or between moulting periods.

The sinus gland is well developed by the time the animal reaches the first adult stage (Fig. 2). It is at this time that it enters the mussel, *Mytilus edulis*, and begins its parasitic form of existence. The most remarkable feature in the subsequent development of the sinus gland is its change of position in relation to the structures of the optic ganglia. In the first stage (Fig. 2) it is found closely appressed to the medulla terminalis; in the second stage (Fig. 3) it has begun to move away from the medulla terminalis. In each successive stage it advances farther toward the distal portion of the eye stalk. In the third stage (Fig. 4) it occupies a position between the medulla interna and the medulla externa, in the fourth stage (Fig. 5) alongside the medulla externa, in the fifth stage (Fig. 6, 21) between the medulla externa and lamina ganglionaris, and in the sixth stage (Fig. 7) it has advanced to a point that is distal to the lamina ganglionaris.

In all stages the sinus gland is found on the dorso-lateral side of the eye stalk. There are very few nuclei in it (Fig. 23) and these bear such a close resemblance to those of the neurilemma, which is continuous with the sinus gland, that one might well consider the sinus gland a modification of the neurilemma (cf.

PLATE III[5]

Microphotographs to show the cyclic phenomena in the sinus gland of *Homarus americanus* and *Cambarus virilis*. All photographs are × 725 reduced about 35 percent, and are of materials fixed in Bouin-Duboscq-Brasil and stained with Foot's modification of the Masson trichrome stain.

FIGURE 12. *Homarus americanus*. A portion of the edge of one lobule of the sinus gland of a specimen fixed forty eight hours before moulting and sectioned at twelve micra. The brilliantly staining acidophilic secretory products are seen as dark irregularly shaped masses. This and succeeding figures show the loose network of connective tissue which constitutes the framework of the gland and the very few scattered nuclei.

FIGURE 13. *Homarus americanus*. A portion of the edge of one lobule of the sinus gland of a specimen fixed six hours after moulting and sectioned at ten micra. The bulk of the secretory masses are only slightly acidophilic and appear less dark in the photograph.

FIGURE 14. *Homarus americanus*. A portion of the edge of one lobule of the sinus gland of a specimen fixed six months after moulting and sectioned at twelve micra. The secretory material is reduced in quantity and stains in a slightly acidophilic manner.

FIGURE 15. *Cambarus virilis*. A portion of the sinus gland of a specimen fixed before moulting and sectioned at nine micra. The numerous brilliantly acidophilic secretory masses are seen as dark masses hung upon the connective tissue framework of the gland. The blood sinus shows as clear areas.

FIGURE 16. *Cambarus virilis*. A portion of the sinus gland of a specimen fixed after moulting and sectioned at nine micra. The secretory products are conspicuous by their absence. The blood sinuses are filled with blood. The nuclei are scattered at random in the loosely arranged connective tissue.

FIGURE 17. *Cambarus virilis*. A portion of the sinus gland of a specimen fixed in December and sectioned at nine micra. The majority of the secretory products present are acidophilic. The blood sinuses appear as clear areas.

[5] All microphotographs were made using Bausch and Lomb microphotographic equipment. The photographs were taken on Eastman Super Panchro Press film, and were printed on Eastman Azo F-2, and Velour Black S-4 paper. Wratten filters G No. 15 and X-1 were used.

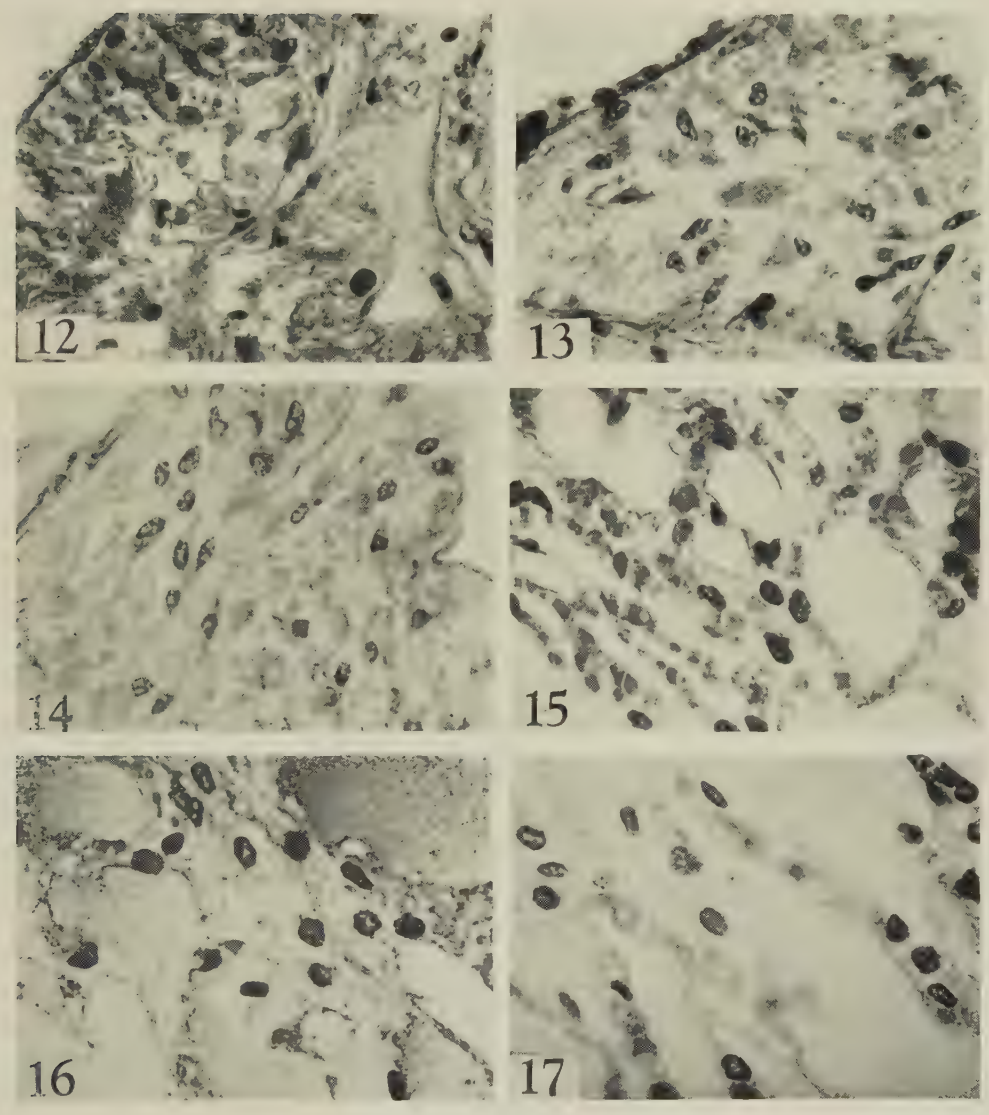


PLATE III

Hanström, 1939). In no stage of the adult is the sinus gland more than partially extruded beyond the level of the neurilemma. In all stages after the second, one finds a very large bundle of nerve fibers passing from the sinus gland toward the medulla terminalis. Some of these pass directly into the neuropile of the medulla terminalis and some ramify among the adjacent ganglion cells. In the first two adult stages the sinus gland is too closely appressed to the medulla terminalis for the presence of the nerve fibers to be readily determined. There is not, however, any evidence that there is a nerve fiber tract which extends directly to the brain as has been reported in Cambarus by Welsh (1941).

There are no obvious cell boundaries in the sinus gland. In fact there are so few nuclei to be found in any particular specimen's sinus gland that the individual cells which constitute the gland must be relatively very large. It is possible to detect canals which extend toward the blood sinus of the eye stalk; presumably these carry the secretory products to the blood stream. The secretions are in the form of large, more or less irregular, masses the amount of which varies very little regardless of the nearness or remoteness of the moulting period.

The secretions of the sinus gland give a basophilic reaction to the stains employed before, after and in the intermoult periods. In specimens fixed while in the process of moulting that portion of the sinus gland which is next to the neuropile of the adjacent optic ganglion gives an acidophilic reaction, whereas,

PLATE IV [6]

Microphotographs to show the sinus gland and X-organ in *Homarus americanus* and *Pinnotheres maculatus*. Figures 18, 19, 20, 22, 23 × 725, and figure 21 × 150; all are reduced about 25 percent. The material shown in figure 18 was fixed in Carnoy-Lebrun, that of figure 20 was fixed in Zenker-formol, and all others were fixed in Bouin-Duboscq-Brasil. The material shown in figure 22 was stained with Mallory's triple stain, and all others were stained with Foot's modification of the Masson trichrome stain.

FIGURE 18. *Homarus americanus*. A portion of the optic ganglion of an embryo fixed in the late egg stage and sectioned at five micra. The arrow indicates the characteristic secretory products of the X-organ which is surrounded by the cell layer of the optic ganglion. (Compare with Fig. 8.)

FIGURE 19. *Pinnotheres maculatus*. Section of the late egg stage embryo showing the position (arrow) of the X-organ. Sections were cut at four micra. (Compare with Fig. 1.)

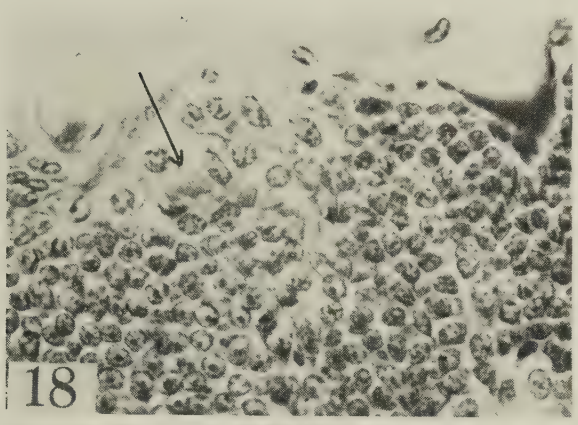
FIGURE 20. *Homarus americanus*. A portion of the eye stalk of a fourth stage embryo, sectioned at seven micra, showing the close association of the X-organ with the cells underlying the eye papilla. The bulge in the exoskeleton can be noted at the top of the photograph. Note that there are fewer nuclei in the X-organ, per unit area, than in the adjacent optic ganglion. (Compare with Fig. 10.)

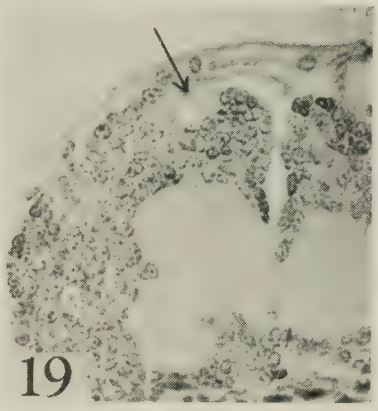
FIGURE 21. *Pinnotheres maculatus*. Section at eight micra of the eye stalk showing the general relationship of the various structures found therein. (Compare with Fig. 6.)

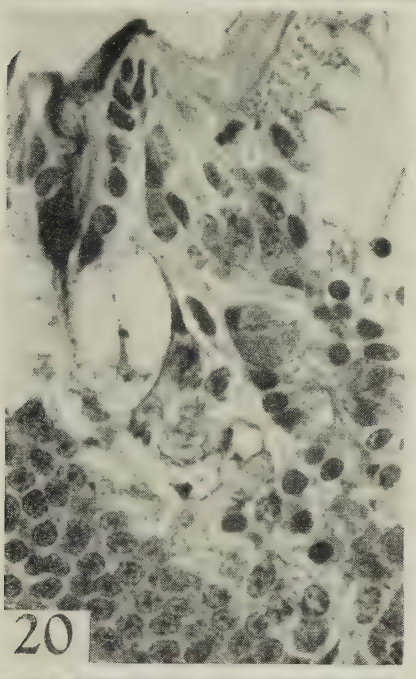
FIGURE 22. *Homarus americanus*. Section of the eye stalk of a third stage after hatching specimen (at seven micra) which shows the sinus gland lying just above the deeply staining muscle. Note that it stains much as the surrounding ganglion does and that the blood sinus is quite small.

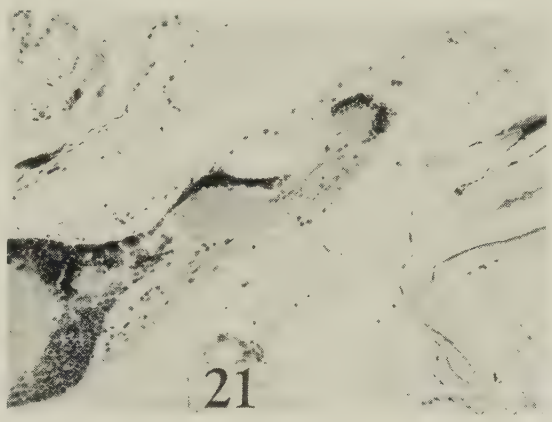
FIGURE 23. *Pinnotheres maculatus*. Section of the sinus gland shown in figure 21 enlarged to show its structure. This is the gland of a specimen that had been starved for forty-six days. Only one nucleus is to be found in this section, and what few secretory products are seen are stained brilliantly acidophilic. Note the indefiniteness to the connective tissue framework of the gland.

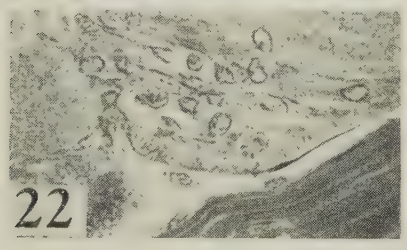
[6] All photographs, excepting that of figure 20, were taken using the same equipment and materials that were used for those of Plate III. The photograph for figure 20 was made on Eastman Ortho-X film using only the X-1 Wratten filter.











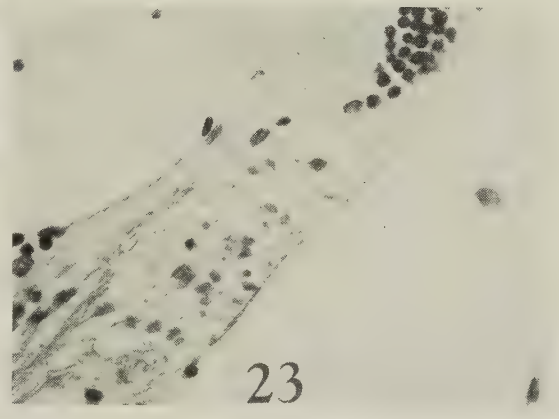


PLATE IV

the lateral portion, next to the blood sinus, gives a basophilic reaction. The acidophilic and basophilic portions blend together in the middle of the gland. Some specimens were starved for varying lengths of time. Those which had been starved for eight days exhibit both an acidophilic and basophilic reaction, but the two reactions are not regionally differentiated as is the case in specimens fixed while in the process of moulting. This reaction is found regardless of whether the specimens are fixed before, during or after the moulting period. In specimens starved for as long as 46 days (Figs. 21, 23) one finds only an acidophilic reaction regardless of the nearness or remoteness of the moulting period. Likewise, as the period of starvation is increased the amount of secretory material present in the gland is decreased although there is no evidence that the decrease due to starvation affects the frequency of moults in this particular animal.

B. *Homarus americanus*

The X-organ is found in the late egg stage (Figs. 8, 18); it is comparable in appearance to the X-organ in Pinnotheres, although there are definite structural differences in it. It is located in that part of the eye stalk that will become the median somewhat ventral side in the first stage after hatching. It is entirely surrounded by the cellular layer of the adjacent optic ganglion, but is separated from the ganglia cells by a thin connective tissue sheath. A definite bundle of nerve fibers extends from the X-organ to the medulla terminalis. The nuclei of the X-organ cells are histologically the same as those of the surrounding ganglia cells: the cytoplasm is more abundant than in the ganglia cells, and that which does not contain secretory products is clear and stains lightly. The secretory products show a series of concentric layers, when sectioned, comparable to those found in Pinnotheres; the nuclei are pushed to one side by the secretory products which nearly fill the entire cytoplasm. In all stages, under low power of the microscope, the X-organ has a similar appearance. This characteristic appearance has been described by Hanström (1939) as a "bunch of grapes." The distal portion of the X-organ extends to the median somewhat ventral portion of the hypodermis of the eye stalk. There is no evidence that there is any eye papilla formed at this time; in later stages the association of the X-organ and the eye papilla is evident. The X-organ exhibits no cyclic phenomena, cytologically, in the egg. The secretory products are always basophilic to the stains employed and vary very little in quantity.

In the first four stages after hatching (Figs. 10, 20) the X-organ increases greatly in size; this is due to the greatly increased number of cells in it and the increased amount of secretory products. Although there is a large increase in the number of cells found in the X-organ there is evidence of only an occasional mitosis after the animal has hatched. At its distal portion the X-organ comes into close contact with the exoskeleton which is bulged at this point. The cuticle of this particular region is extremely thin; this is the eye papilla (Figs. 10, 20). The eye papilla cells are found on the distal side of the X-organ between it and the ommatidia of the eye. There is no connection between the X-organ and the eye papilla as the X-organ is completely surrounded by a connective tissue sheath. No bipolar cells are found in the distal portion of the X-organ that are comparable to those described by Hanström (1937, 1939) for the adult of *Homarus*

americanus. Cytologically there is no evidence of any cyclic phenomena in the X-organ during the first four stages after hatching; the secretory products are basophilic and the quantity is quite constant.

In the adult (Fig. 11) the X-organ no longer extends to the exoskeleton, but is found in the proximal half of the eye stalk. The basal portion of it is imbedded in the cellular layer of the distal part of the medulla terminalis. The distal portion extends well beyond the ganglionic cellular layer (to a point approximately level with the distal end of the medulla interna) into the blood sinus of the eye stalk. The X-organ occupies the same general position that it occupies in the earlier stages. In general structure it has become considerably more complex; it is now divided into a large number of units each of which is composed of from ten to twenty or more cells. Each of these units has a circular, whorled appearance. The nuclei are arranged around the periphery and the secretory products occupy the central area. A large bundle of nerve fibers passes around each of the units giving off nerve fibers to the individual cells. This arrangement gives the serial sections an appearance of being a series of whorls each of which originates from a common central stem of nerve fibers. The main bundle of nerve fibers passes between the various units and extends to the median side of the medulla terminalis. The nuclei have increased prodigiously in numbers, but still bear a marked resemblance to those of the cells of the optic ganglia. The cytoplasm of the X-organ cells is large and irregular in shape; it is filled for the most part with secretory products which have the characteristic concentric layers within them. Cells not possessing secretions have a clear lightly staining cytoplasm.

There is no evidence of cyclic phenomena associated with moulting as far as the X-organ is concerned. The basophilic reaction is found regardless of whether the eye stalk has been fixed a few days, 48 hours, six hours before, six hours, 48 hours, one, one and one-half, four or six months after moulting. Likewise, there is little change in the amount of secretory products that are evident in the X-organ of the eye stalks in the above series; the number of blue staining concretions is remarkably constant. In the case of the specimen that had not moulted for more than one year there were fewer secretory products present and more of the units contained vacuoles.

As far as can be determined the sinus gland is not formed sufficiently to be definitely recognized as such until the third stage after hatching (Figs. 9, 22). At this time it is a thin, lightly staining structure located on the dorso-lateral side of the eye stalk between the medulla interna and medulla externa. It is not very conspicuous as it does not give the typically brilliant acidophilic reaction to acid fuchsin that is found in the adult sinus gland. Structurally the sinus gland has the appearance of being a thickened portion of the neurilemma which invests the optic ganglia. The nuclei are few in number and stain precisely in the same manner as the nuclei of the neurilemma. The cell boundaries cannot be discerned; the cytoplasm seems to be confined to the connective tissue framework of the gland upon which the secretory materials are hung. The general tissue of the gland, regardless of what it is composed, stains very lightly with all the stains employed. There is a definite nerve fiber tract which extends from the sinus gland to the lateral distal border of the medulla terminalis. It is this fact that makes it possible to ascertain the presence of the sinus gland in the third

stage after hatching. No such innervated structure has been found in the earlier stages.

In the fourth stage after hatching (Fig. 10) the eye stalk has increased more in thickness than in length. Consequently, the medulla interna is displaced; the sinus gland is found on the proximal portion of the medulla externa lateral to the medulla interna. This brings the sinus gland into closer proximity to the medulla terminalis. The sinus gland has increased in size with the resultant increase in the number of nuclei found in it, but the cytoplasm is still lacking the brilliant acidophilic reaction one might expect. No cell boundaries are visible; the nerve tract from the medulla terminalis is much more prominent than in the third stage after hatching.

In the adult (Fig. 11) the sinus gland occupies the same general position as in the early stages, but the eye stalk has become much more extended so that the medulla terminalis, interna and externa and lamina ganglionaris are strung out and occupy a much smaller portion of the inside of the eye stalk than they did in the early stages. As a result the sinus gland is found in the proximal half of the eye stalk on the opposite side from the X-organ. It is much more highly developed and extended than in the early stages. Situated alongside the medulla interna and extending to the proximal portions of the medulla externa it sends large finger-like processes out into the adjacent blood sinus. The nerve fiber tract extending from the sinus gland to the medulla terminalis is very large; after the protargol stain of Bodian (1937) one finds that the nerve fibers ramify among the fibers of the neuropile of the medulla terminalis and branch to all parts of the sinus gland. The framework of the gland is composed of connective tissue which stains precisely the same as the other connective tissue found in the eye stalk. There are no distinct cell boundaries observable in most preparations, but occasionally one is able to find an isolated cell which has a definite cell boundary surrounding a large irregular cytoplasmic mass. The nuclei have the same appearance as those of the early stages; they look more like connective tissue nuclei than nerve cell nuclei.

As has been pointed out above there is no striking staining reaction in the sinus gland of the third and fourth stages after hatching. In the adult, however, there are some interesting phenomena. In the series obtained for this research the following reactions are discernible: Specimens fixed several days and a few hours before moulting have the sinus gland filled with irregularily shaped secretory granules (Fig. 12) which, after Foot's modification of the Masson trichrome stain and other stains employing acid fuchsin and aniline blue, give a brilliant acidophilic reaction for the most part although there are a very few granules which react basophilically. Specimens fixed six hours, 48 hours, one and one and one-half months after moulting give three characteristic reactions. Some of the granules are brilliantly acidophilic, some are slightly acidophilic, and a number are decidedly basophilic (Fig. 13). In specimens fixed four and six months after moulting the amount of secretory material in the sinus gland is decidedly less than in those fixed during the summer months at or near the time of moulting (Fig. 14). In these cases the secretions are for the most part only slightly acidophilic with an occasional basophilic granule being found. In the specimen that had not moulted in over a year there was less secretory material in the sinus gland than was found in those (fixed in the summer months) which had moulted,

but there was more than was found in those specimens fixed in the late fall and winter. The secretory material was brilliantly acidophilic, slightly acidophilic and basophilic. There was more basophilic material in this particular specimen than in any of the others. Examination of the exoskeleton showed that a new exoskeleton had been laid down underneath the old one which had not, for some reason, been shed.

C. *Cambarus virilis*

When the cyclic phenomena were found in the sinus gland of Homarus it was thought advisable to study the sinus gland of *Cambarus virilis* in which Dethier (1942) had previously reported a similar reaction. Accordingly, sections were made of the eye stalks of specimens fixed just before and just after moulting as well as of those fixed in late December. The sinus gland of specimens fixed just before moulting (Fig. 15) was filled with many irregularly-shaped granules which for the most part gave a brilliant acidophilic reaction, but there were occasional granules which were basophilic. In those specimens fixed after having completed moulting (Fig. 16) there was a sharp reduction in the number of secretion granules present; a few of these were brilliantly acidophilic, but most of them exhibited varying degrees of a basophilic reaction. In those specimens fixed late in December (Fig. 17) there were about the same number of granules as were found in the post-moult specimens, but the majority of these were acidophilic and only a few basophilic.

Discussion

Dethier (1942) in her account of the sinus gland in Cambarus states that she has been able to trace it from the first post-embryonic moult, and that it is apparently functional at that time.[7] This is not the case in the two species used for this investigation; in Homarus it has been impossible to ascertain definitely its presence until the third stage after hatching, and in Pinnotheres it could not be detected (with the techniques used) in the egg or first zoeal stages. Cytologically the evidence seems to indicate that the sinus gland in the third and fourth stages of Homarus is not a functional gland.

It has fairly well established that the color changes in Crustacea are controlled by hormones which originate in the eye stalk. As Kleinholz (1942) points out "the glandular tissue is probably the sinus gland, although the X-organ may also be concerned in this function." The apparent absence of the sinus gland in the early stages suggests that the X-organ may be functional in this capacity at this time, but the cytological evidence does not bear this out in Homarus and Pinnotheres. On the other hand, Cambarus has no X-organ which has the characteristic concretions of secretory material that are comparable to those found in Homarus and Pinnotheres. (Welsh, 1941, has found a mass of tissue on the dorso-lateral side of the medulla terminalis which he suggests may be the X-organ in *Cambarus bartoni*.)

Megušar (1912), Abramowitz and Abramowitz (1938, 1940), Brown and Cunningham (1939), Kleinholz and Bourquin (1941), and Smith (1940) have

[7] When the crayfish hatches it is a miniature adult with all appendages etc., and is comparable to a fifth or sixth stage of *Homarus americanus*.

shown that the removal of both eye stalks from crustaceans hastens the onset of moulting. Smith showed quantitatively that the removal of both eye stalks shortened the intermoult period by slightly more than 30 per cent. This probably indicates that some structure in the eye stalk, possibly the sinus gland, produces a hormone which has an inhibiting effect upon moulting. Kyer (1942) gives good evidence that the sinus gland, when active, specifically inhibits moulting and gastrolith formation. Dethier (1942) in her account of Cambarus suggests that there is an acidophilic basophilic series which is related to the period of moulting. In the cases of Homarus and Cambarus the acidophilic reaction before moulting and the basophilic one after moulting seem to indicate cyclic changes in the sinus gland which are directly related to the moulting process. Further evidence of the activity of the sinus gland is exhibited by the reduction in the amount of secretory material in it; this is most striking in Cambarus, less evident in Homarus and scarcely detectable in Pinnotheres (this is probably due to the fact that Pinnotheres passes through several moults in fairly rapid succession).

The explanation of the basophilic and acidophilic reactions in Pinnotheres is more difficult on the basis of secretory activity. If one had only the normal animals to consider it might be possible to state that the activities of the sinus gland in this species passed through a reverse acid-base reaction which were a direct result of its activity. However, in as much as the sinus gland of the starved animals, and that in the ones in the process of moulting, both give acidophilic reactions it may be that the lack of food changes the pH- of the sinus gland from a normally basic range to an acid range. Since the animal does not feed during the period of ecdysis this may account in part for the acidophilic reaction of the sinus gland at this time.

Plankmann (1935) reported that various factors (starvation, etc.) may affect the rate of moulting. The Pinnotheres that were starved for varying periods of time were kept at a temperature comparable to that of their normal environment, on a dark background and in running sea water. There was no increase in the frequency or number of moults that occurred; it was the sinus gland that showed the affect of starvation and the X-organ appeared unchanged.

In the case of retinal pigment migration Parker (1897) could find no nerve fibers supplying the distal pigment cells in Palaemonetes. This observation started the controversy of the interrelationship of the eyes and subsequently many investigations have been made upon this subject. It has been shown in a generally satisfactory manner that the sinus gland produces a retinal pigment hormone (cf. Welsh, 1941). The question is raised as to the mechanism involved in the early stages where there is no obvious sinus gland to be found. If the sinus gland is the sole controlling factor it must be assumed that the early stages are incapable of retinal pigment migration.

Further studies are necessary to give satisfactory answers to the following points which have not been completely answered in the present study:

1. From precisely what pre-existing tissue is the sinus gland formed?
2. Is the sinus gland a syncytium?
3. Is the sinus gland noncellular and merely a storage space or are there cells which periodically fill with secretory products and break down (e.g. is secretion holocrine?)?

Summary

1. The histogenesis of the sinus gland and X-organ have been studied and described for the egg, first zoea and adult stages of *Pinnotheres maculatus*.

2. The sinus gland is not found in the egg or first zoea, but it is found in all the adult stages of Pinnotheres.

3. The X-organ is found in the egg and other stages of Pinnotheres.

4. The histogenesis of the sinus gland and X-organ have been studied and described for the egg, first four stages after hatching and the adult of *Homarus americanus*.

5. The sinus gland is not found as a definitely discernible structure in Homarus until the third stage after hatching.

6. The X-organ is found in all stages of Homarus that have been studied.

7. Evidence is presented for the existence of cyclic secretion phenomena in the sinus gland of all species studied.

8. There is no evidence of the existence of cyclic secretion phenomena in the X-organ in any of the species investigated.

Acknowledgments

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Contribution No. 327

LIFE ACTIVITIES OF FORAMINIFERA IN RELATION TO MARINE ECOLOGY

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ABSTRACT

The number of chambers in the test of a foraminifer is indicative of the degree of maturity of the individual, and the rate of growth can be determined from the mean number of chambers in a series of samples of a population. The presence of juveniles in a sample and cytological evidence of gametogenesis suggest periods of reproduction. From the number of generations produced annually, the number of individuals asexually produced as determined from cultures, and the ratio of microspheric to megalospheric individuals on the floor of the sea, it is possible to estimate the productivity of a species. The rate of mortality from various causes and at different seasons can be estimated from the number of living Foraminifera per unit area on the floor of the sea. When these data are correlated with data concerning measurable ecological conditions in the sea, it is possible to evaluate the relative importance of these and other conditions on the success of a species. When frequency-distribution curves for growth stages in populations, taken in a region where the conditions affecting growth and reproduction have been studied, are compared with similar curves for samples of populations taken in a region where measurements of these conditions have not been made, much may be deduced concerning the possible seasonal variations in these conditions in the second region. *Elphidium crispum* (Linnaeus) was used in this work, and the conditions discussed include food, bottom, depth, temperature, salinity, pH, oxygen tension, turbulence, turbidity, animal associates, parasites, and enemies. Series of samples were taken in the vicinity of Plymouth, England; Nice, France; Batavia, Java; and La Jolla, California.

INTRODUCTION

THE basis for interpretative work in paleoecology is knowledge of those conditions which are essential to the success of species similar to those observed in fossiliferous deposits. The persistent nature of the calcareous tests of the Foraminifera, their universal distribution in marine sediments, both recent and geological, and the notable effect of slight differences in ecological conditions upon the species in populations make them a useful guide to the biotic conditions that existed in ancient seas. Thus far, papers on the ecology of the Foraminifera have consisted of descriptions of assemblages of presumably empty tests, and the only chemical and physical conditions in the sea that have received more than a passing comment have been temperature, depth, salinity, and bottom sediments (see Vaughan, 1940, and Ladd, 1941).

In our study of the productivity of the Foraminifera in the sea (Myers, 1942*a*), the life span, rate of growth, rate of reproduction, population per unit area, and the rate at which the tests are contributed to the sediments were discussed. In the present paper we shall attempt to correlate these data with measurable ecological conditions in the sea, including temperature, salinity, oxygen tension, pH, illumination, and the nutrient salt content of the water as well as the influence of a varying food supply upon growth and test morphology, the effect of the substratum upon distribution, and the importance of animal associates, parasites, and other enemies.

Further and more exact laboratory experiments would have contributed additional infor-

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mation concerning the limits of tolerance of the Foraminifera for certain ecological conditions, but could only have supplemented the interpretation of the observed effect of these variable conditions acting simultaneously upon the several phases of the life cycle. Our purpose in this and the preceding paper has not been to make arbitrary statements where the evidence did not warrant, but rather to present observations and suggest methods that would contribute to a better understanding of the role of the Foraminifera in the economy of the sea.

Material and Methods

Elphidium crispum (Linnaeus) was used for the quantitative study of the productivity of the Foraminifera referred to above. The present paper makes use of the same data in an attempt to explain the influence of ecological conditions in the sea upon the life activities of the same species.

When individuals of *E. crispum* are killed, hardened, decalcified, stained, and cleared, it is possible to distinguish those that were feeding actively, were about to reproduce, or were in a dormant condition. When chamber counts for samples of populations taken monthly for twelve months and numbering from 500 to 1,000 individuals are plotted as percentage frequency-distribution polygons, much may be learned concerning the rate of growth, the life span, the periods of reproduction, and the influence of ecological conditions upon the life activities (see plate I, figs. 1 and 10).

In the life cycle of *E. crispum* there is an orderly succession of sexual and asexual generations. This alternation of generations results in microspheric individuals having a minute initial chamber and megalospheric individuals in which the initial chamber is relatively enormous. Microspheric agamonts asexually produce megalospheric gamonts, which in turn give rise to biflagellated gametes. The union of pairs of gametes in fertilization results in zygotic amoebulae that develop into microspheric agamonts. Reproduction terminates the life of the individual because all of the protoplasm and much of the calcium carbonate in the test is utilized in the production of the new generation or the gametes, as the case may be. The sexual phase of the life cycle is least successful as regards the number of progeny produced, with the result that during much of the year the ratio of sexually produced microspheric to asexually produced megalospheric individuals living in tide pools is only about 1:25, while below low-tide level the ratio is 1:40 or even 1:50. Immediately after sexual reproduction by megalospheric gamonts in the spring, ratios as low as 1:1 have been observed. The former ratios are again restored following asexual reproduction by the microspheric agamonts (Myers, 1942*a*).

Much of the material for this and the preceding paper was taken within Plymouth Sound, on the southwestern coast of England, where the average depth does not exceed 30 feet and the extreme tidal range is 16 feet (fig. 1). Several streams

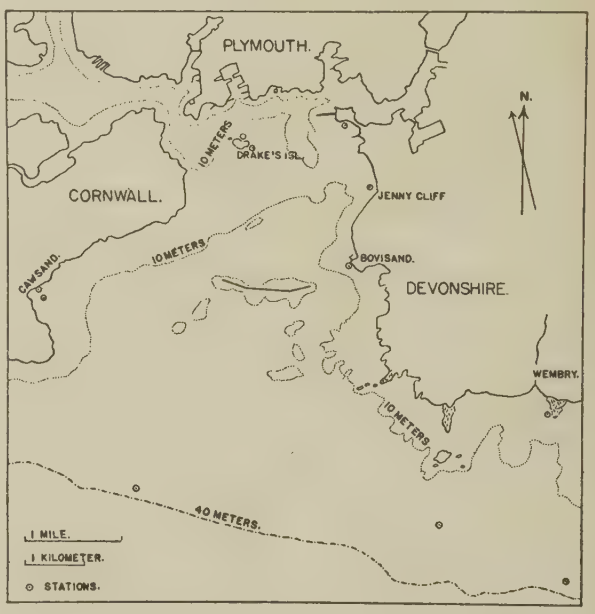

Fig. 1. Map of Plymouth Sound showing the position of the stations referred to in the text.

with wide estuaries empty into the sound, and during periods of heavy rainfall these affect the salinity, particularly at the surface, where it varies with each tidal phase, and from season to season. The estuaries and the main channel have mud bottoms, while on either side of the main channel where the Foraminifera are most numerous, the bottom is covered with quartz sand and fine shell gravel. The abrupt rocky shores are fringed with dense growths of *Fucus*, *Laminaria*, and other algae, and tide pools on the shelving reefs usually contain dense growths of *Corallina*. The region supports a rich and varied macrofauna and microfauna, including the densest population of *E. crispum* we have ob-

served in any sea or ocean. Additional material from the English Channel in the vicinity of Plymouth Sound as well as material from the north coast of the Mediterranean Sea, the western end of the Java Sea, and the Pacific coast of California will be discussed.

The methods used for collecting and preparing the Foraminifera for study have been amply described (Myers, 1942*a*), and for that reason will not be repeated here.

In dealing with populations of larger invertebrates such as mollusks, echinoderms, and arthropods, migrations and the segregation of age groups according to depth or other conditions are often confusing (Moore, 1938–1939). In the Foraminifera the rate of movement is too slow to account for any of the changes in populations discussed, for when sediments or washings from algae containing *E. crispum* are placed in an aquarium, the more active individuals work to the surface of the sediments and up the walls to a height of 9 to 12 cm. in 12 hours. This movement is mainly in one direction and probably approaches the maximum rate at which these organisms are capable of moving over the floor of the sea. One of these Foraminifera moving continuously at this rate for one month would traverse a distance of only 7.2 meters. In one region we obtained samples of a population of *E. crispum* that had been sorted according to size as a result of winter turbulence and current action, but such an occurrence is unusual.

Food of the Foraminifera

The Foraminifera, in common with other free-living rhizopods, are holozoic, that is, they depend upon particulate food for sustenance. Cytoplasmic inclusions in the terminal and adjacent chambers in the majority of benthonic species living in shallow water consist for the most part of unicellular plants derived from the substratum, and to a lesser extent the phytoplankton in the overlying water. Diatoms are the most numerous and readily identifiable inclusions, although soft-bodied flagellated organisms, including the gametes of larger algae, filamentous algae, minute eggs, and cysts, are also recognizable. Feeding experiments and the rare inclusion of an appendage of a minute arthropod in the distal chamber of a test suggest that the Foraminifera digest animal tissue by means of ferments carried in the pseudopodia; and in Foraminifera in which the aperture of the test will admit sizable particles, digestion continues after the food has been ingested. Although most Foraminifera readily accept animal tissue as food, those which we have studied, including larger species found only in tropical waters, seem incapable of capturing the smallest ciliate or the minute nauplii of arthropods that become entangled in the filose and anastomosing pseudopodia when zoöplankton is added to well-established cultures in which the Foraminifera are actively feeding upon other organisms. Therefore it would appear that the main sources of animal proteins available to the Foraminifera consist of dead organisms or the residual protoplasm contained in sloughs and exoskeletons (pl. I).

The pseudopodia of *E. crispum* are attenuated to the vanishing point except when viewed under the highest powers of the microscope, and it is exceedingly doubtful whether this species captures organisms capable of offering more than passive resistance. Because of the small diameter of the apertures of the test, the only recognizable food inclusions are soft-bodied organisms, presumably the gametes of algae (Lister, 1895; Føyn, 1936) and minute fragments of diatom frustules. Material recently removed from the sea often contains tests of *E. crispum* to which are attached masses of ectoplasm in which are enmeshed numbers of minute organisms and bits of organic debris upon which the Foraminifera were probably feeding—a condition that was most conspicuous during the spring outburst of phytoplankton production. Although we have less direct evidence as to the nature of the food of *E. crispum* than we have for species in which entire organisms are ingested, it seems evident that the food of this species, in common with other Foraminifera, includes both micro plants and animals, either living or dead, and derived both from the water and from the substratum upon which they live.

Before we consider the possible influence of other ecological conditions upon the life activities of the Foraminifera in the sea, it is necessary to consider the probable effect of the seasonal distribution of food organisms upon growth and reproduction. For a discussion of the food of the Foraminifera, see Jepps (1926), Calvez (1938), Føyn (1936), and Myers (1935, 1936, 1940, and 1942*a*).

Food Cycle of the Foraminifera in the Sea

Foraminifera as well as other animals living in the sea depend directly or indirectly upon the utilization of nutrient salts by plants. For a

time these salts are bound up in the substance of the plants and the animals that eat them, but they are again restored to the water following death and bacterial decomposition. In the vicinity of Plymouth Sound the maximum concentration of nutrient salts is to be found in the early spring, and with the lengthening hours of sunlight in March there occurs an outburst of photosynthetic organisms, principally diatoms, which rapidly reduce the concentration of these salts, so that by June the available supply of nitrates is largely depleted, and there is almost a complete absence of soluble phosphates (fig. 2). Where the depth of the water does not exceed that of the photic zone, there develops on the bottom a microflora, the productivity of which is controlled by the same conditions that limit the productivity of the phytoplankton, and along the shore these same conditions also determine the rate of growth and reproduction in the larger algae whose spores are used as food by the Foraminifera. The spring crop of phytoplankton soon depletes the nutrient salt content of the water, and in turn the phytoplankton is depleted by a rapidly increasing population of herbivores, whose consumption of these plant cells is more dependent upon the number of cells available than upon the actual requirements of the animals, since the number of faecal pellets observed in the water bears a closer relationship to the number of diatoms in the sea than to the zoöplankton population. These faecal pellets, rich in nutrient salts, provide a suitable substratum for the growth of bacteria, and at depths appreciably below the photic zone these faecal pellets and their bacterial flora probably constitute an important source of food for the Foraminifera.

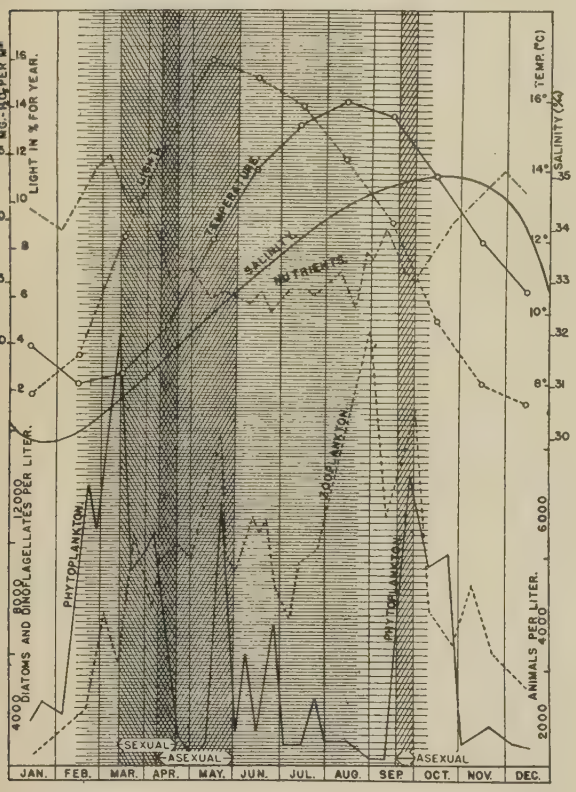

FIG. 2. Ecological conditions in the sea in relation to growth and reproduction in *Elphidium crispum* living on a sandy bottom 9 meters below low-tide level. The curve for nutrient salts based on the P_2O_5 content of the water would roughly parallel curves for NO_3 and SiO_2, and the curves for plankton organisms show the abundance of individuals but do not take into account the numbers produced or utilized by plankton feeders. Growth and reproduction in the Foraminifera as indicated by shading were most pronounced in the spring when food was abundant and at a time when other conditions might be considered least favorable, and under conditions that except for food were vastly different from those which induced reproduction in the fall. In the late fall conditions related to storm-induced turbulence greatly reduced the activity of the Foraminifera, while food was still abundant and other conditions seemed favorable for further growth.

After the spring outburst of phytoplankton there are several minor summer outbursts, and a longer and less intense fall outburst, which is limited by the decreasing hours of sunlight as winter approaches. The abrupt decline in the phytoplankton population in the spring does not result in a corresponding reduction in the numbers of plankton animals, so that variations in the zoöplankton populations are neither so frequent nor so intense. Temperature seems to have slight influence upon phytoplankton production, for February and March, the months of greatest abundance, include the period of lowest water temperatures in the sea, while in September and October, the months that are second in abundance, the water is only slightly colder than in August, the warmest month in the year. For references and a more complete discussion of plankton production in the sea, see Harvey et al. (1935).

The plankton cycle within Plymouth Sound is influenced by somewhat wider fluctuations in hydrographic conditions than in the English Channel opposite Plymouth, where the data for figure 2 were obtained; but from the available data and the amount of water exchange, it is evident that the dominant features, including a spring and fall

outburst of phytoplankton production and a more gradual increase in the zoöplankton, beginning in the spring and reaching maximum in the early fall, are similar in the two areas. The time and intensity of these outbursts vary somewhat from year to year, and the curves presented in figure 2 should be considered with this in mind.

Influence of Food upon Growth and Test Morphology

In Plymouth Sound the diameter of the proloculus of microspheric tests in samples taken from tide pools ranges from 6μ to 16μ, while those taken in the sublittoral zone range from 6μ to 19μ. The diameter of the proloculus of megalospheric tests from these life zones is 28μ to 73μ and 54μ to 125μ, respectively. The rate at which chambers are added to the tests depends upon the need for growth that results when food is consumed in excess of the basic requirements of the organisms. When adequate food is available, growth is continuous; therefore the number of chambers produced is related to the duration of the period when sufficient food is available to induce growth. The mean number of chambers produced by microspheric agamonts in the littoral and sublittoral zones during the first growing year was 29 and 48, respectively, while in megalospheric gamonts it was 21 and 34. This amounts to 40 percent more chambers and a 60 percent larger test for individuals produced in the sublittoral zone compared with those from the littoral zone—a difference that had persisted from the earliest developmental stage (figs. 3 and 5, and Myers, 1942*a*).

It might be argued that these two groups of *E. crispum* which are similar morphologically are dissimilar physiologically as a result of genetic differences. This could be demonstrated by maintaining cultures of pure strains of *E. crispum* for several generations under controlled conditions. However, it is obvious that growth is greatest where food is most abundant and where feeding activities are not interrupted by adverse conditions that result from surf and tidal changes.

The tests of *E. crispum* also vary in shape, depending upon the ecological conditions under which they are produced (fig. 4). In the spring, when food is abundant, the chambers added to the test are longer, narrower, and thinner than those added the previous year, especially in the late fall, when food is relatively sparse. For this reason there is often an abrupt break in the series of chambers, which results in the test having a notched keel (fig. 4*C*). Variations in the length of the chambers are most noticeable in the protoplasmic casts of the Foraminifera, and these were used in determining the rate at which chambers are added to the tests in the early months of the second year.

The maximum rate of growth and the largest tests were produced in the western part of Plymouth Sound below the Jenny Cliffs, the second largest opposite Drake's Island, and the smallest opposite the town of Cawsand, where conditions were least favorable (fig. 1). When the rate of growth is retarded, the test is thicker and more circular in outline (fig. 4*B*), while rapid growth results in a decidedly thinner test, a more distinct keel, and a terminal chamber having a longer apertural face (fig. 4*A*). This thinner type of test was the only one produced in the vicinity of Drake's Island in 1939, while thicker tests from less favorable years were numerous in the surface sediments of this area. In tropical regions, where the rate of growth is somewhat less but more continuous, the tests of this and related

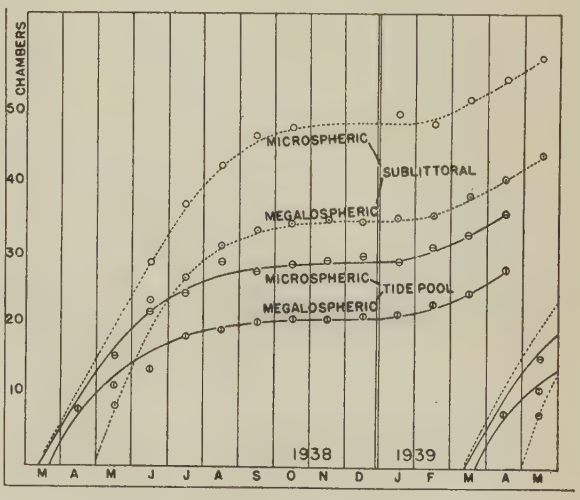

Fig. 3. Rate of growth in *Elphidium crispum*. Entire lines represent tide-pool populations, and dotted lines populations in the sublittoral zone. Growth during the first year is based on the mean number of chambers in the samples and for the spring of the second year on the mean number of chambers added to larger tests since reproduction by larger individuals tends to reduce the mean size. Sexual reproduction as determined from cytological evidence of gametogenesis began at about the same time in both life zones. Asexual reproduction in tide pools began about 1 week later and below low-tide level about 5 weeks after that. Forty percent more chambers and a 60 percent larger test were produced in the sublittoral zone.

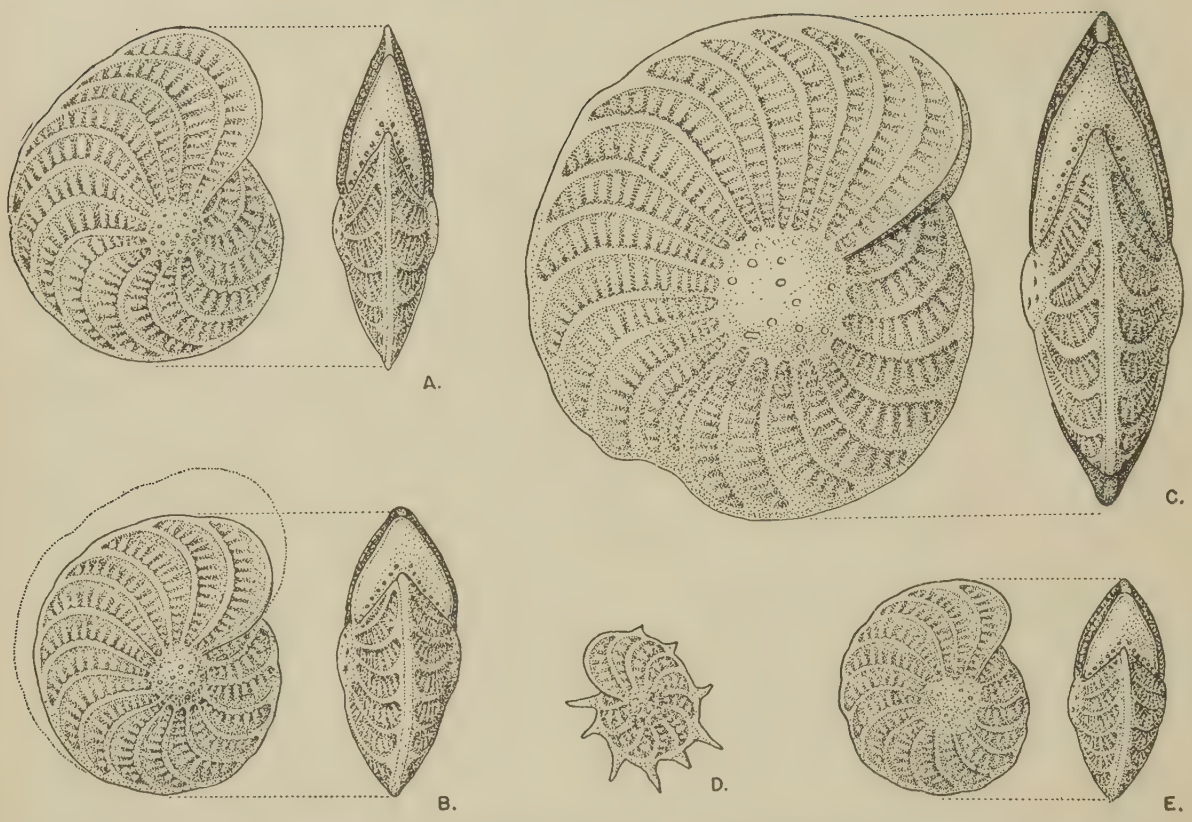

FIG. 4. Tests of *Elphidium crispum*. *A*. Juvenile with long narrow chambers resulting from rapid growth induced by an abundance of food. *B*. Juvenile with shorter, wider chambers and a more circular outline, the result of less favorable feeding conditions. *C*. Mature individual showing an indentation in the margin of the test marking the limit of growth prior to the period of winter dormancy. *D*. Juvenile with spines. *E*. Mature individual from a tide pool where the rate of growth is comparatively slow. *B* would seem to be an intergrade between *A* and *E*, although there is a difference in the texture of the tests from the two zones that cannot be shown in drawings. Based on camera lucida drawings × 625.

species are thicker than those produced in higher latitudes and are often regarded as distinct species. *E. crispum* is universally distributed in coastal waters except for areas within the polar seas, and if slight variations in the shape and texture of the tests which result from the conditions under which they are produced were to be considered adequate to justify the erection of new species and subspecies, the group could be subdivided indefinitely. However, slight differences in test morphology have a decided value to the stratigrapher.

Influence of Food upon Growth and Reproduction

In the littoral zone the plankton content of the water that ebbs and flows with each tidal cycle is grazed upon by myriads of animals living at all levels upon the beach; therefore the food of the Foraminifera living in this life zone is limited for the most part to the microflora produced in the substratum upon which they live. The tidal flux also exposes these Foraminifera to abrupt changes in environmental conditions, and their success depends in part upon their ability to make rapid readjustments to a changing environment. In the sublittoral zone, where ecological conditions are more stable, tidal currents and a considerable vertical mixing of the water assure the Foraminifera a maximum amount of food in addition to the microflora living on the bottom, and we have found that these differences in ecological conditions, including the relative abundance of food in the two life zones, are responsible for the fact that in all latitudes populations of Foraminifera living between high- and low-tide level have few species in common with populations living below low-tide level, although as-

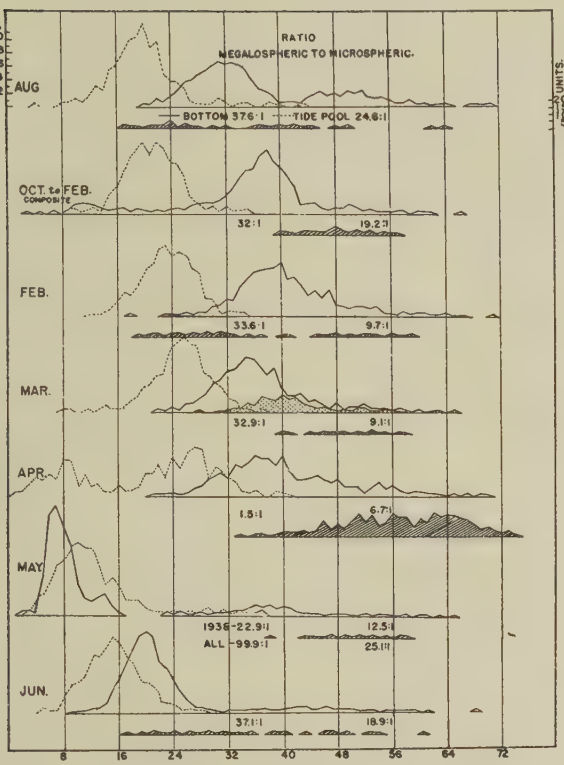

FIG. 5. Percentage frequency-distribution curves for growth stages in *E. crispum* based on the number of chambers in samples of populations. Broken lines represent samples of 500 megalospheric individuals taken in tide pools, and entire lines, 1,000 megalospheric individuals taken in the sublittoral zone. The curves that are cross-hatched are for microspheric individuals in the latter samples, and the stippled area in the curve for March is for megalospheric individuals showing cytological evidence of gametogenesis.

semblages of empty tests of Foraminifera found in sediments taken near the coast include those of species common to both zones. After periods of storm-induced turbulence some mixing of populations takes place, but individuals usually fail to survive for long when removed from their natural habitat to a new environment.

Ecological conditions other than food are probably responsible for the observed distribution of *E. crispum* within Plymouth Sound, and the enormous numbers of individuals in restricted areas within each of the two life zones suggest that this species is well adapted to the conditions within the restricted areas. However, in the sublittoral zone the rate of growth in *E. crispum*, as we have said, is 40 percent greater and the diameter of the test is 60 percent larger than in the littoral zone, and it would seem that this difference in growth is an expression of the relative abundance of food available to these two groups of Foraminifera. This is in agreement with the observed diminution in size of individuals of other species of Foraminifera produced in cultures in which there was a sparsity of suitable food organisms (Myers, 1936).

Winter is a period of dormancy, for both the Foraminifera and the organisms upon which they depend for food, as is evident from the sparsity of food organisms in the distal chambers of the tests and the amount of shrinkage in the protoplasmic content of the earlier-formed chambers (Myers, 1942*a*). After a rapid increase in the numbers of photosynthetic organisms in the plankton in February, thin sections of most species of Foraminifera show enormous numbers of diatoms and other unicellular plants. At about the same time the distal chambers of *E. crispum* contain comparable numbers of the gametes of larger algae, and the protoplasmic content of the earlier-formed chambers shows less and less shrinkage. An increase in metabolic activities results in growth through the addition of new chambers to the test, and after two to four chambers have been added, megalospheric individuals that are at least half-grown produce gametes. Gametogenesis marks the beginning of an intense period of sexual reproduction, which persists for three to four weeks. Near the end of this period vast numbers of juvenile megalospheric individuals are to be found in the sediments, indicating that asexual reproduction by individuals of the larger microspheric generation is taking place. In figure 2 it will be observed that growth, sexual reproduction, and asexual reproduction follow in sequence, and that this period of unusual activity on the part of the Foraminifera coincides with the spring outburst of phytoplankton production. Although the phytoplankton is only one source of food available to the Foraminifera, its relative abundance is probably a dependable index to the productivity of the more important microflora in the substratum upon which benthonic Foraminifera live and take much of their sustenance, because the productivity of both groups of food organisms is largely dependent upon illumination and the nutrient salt content of the water.

INTERPRETATION OF FREQUENCY-DISTRIBUTION CURVES OF GROWTH STAGES

Percentage frequency-distribution curves for growth stages based on the numbers of chambers

in 500 megalospheric individuals in samples of populations taken in tide pools show but a single mode for 11 of the 12 months (fig. 5). Beginning in April, the mode shifts rapidly in the direction of increasing size until August, and then at a diminishing rate until November, when growth is discontinued until the following March. The curve for April has two distinct modes, the first representing a new generation that had been asexually produced by microspheric agamonts, and the second, those larger individuals which had survived from the previous year, and which were about to produce sexual gametes.

A set of curves similar to those discussed above was also prepared for individuals of the microspheric generation in the same populations. From these curves, together with the changing ratios of microspheric to megalospheric individuals, it is evident that sexual reproduction takes place in March, or about one month earlier than asexual reproduction, and there is ample cytological evidence to confirm these dates (Myers, 1942*a*).

When the growth stages in megalospheric individuals in samples taken in the sublittoral zone are plotted in a like manner, the curves for April through September have two distinct modes (fig. 5). From this it is evident that from 10 percent to 22 percent of the population of megalospheric individuals fail to reproduce at the end of the first year and continue to live through the second year. It is also evident that although sexual reproduction takes place at about the same time in both life zones, asexual reproduction occurs in May, or about one month later than in the littoral zone. In the sublittoral zone a second period of asexual reproduction occurs in September, and it is significant that this second period of asexual reproduction also coincides with an intense outburst of phytoplankton production (fig. 2).

No corresponding period of asexual reproduction takes place in the littoral zone, where a shortage of food no doubt results from the feeding activities of an enormously increased population of animal associates compared with that of the previous spring. From these data it is evident that the life span for individuals of each generation is usually one year, and that in the littoral zone only two years are required to complete the life cycle, while in the sublittoral zone reproduction is delayed in a portion of the population until the second year, and three or even four years may be required to complete the life cycle.

Drake's Island.—We know that the shape of the curve representing growth stages in a tide-pool population of *E. crispum* taken on Drake's Island in August was influenced by an intense outburst of phytoplankton in the spring, and that most individuals reproduced within a relatively brief period. This accounts for the few classes of growth stages represented and the abrupt nature of the curve (fig. 6). From a series of curves we have determined the maximum number of chambers in tests of this species, the length of the growing season, and the rate of growth (fig. 5). Knowing the time, intensity, and duration of the periods of growth and reproduction in *E. crispum*, we can deduce much concerning the intensity and duration of the major outburst of phytoplankton production from a single frequency-distribution curve of growth stages in a sample of a population taken in a region where little or no data are available concerning the phytoplankton cycle in the sea, as we shall endeavor to explain.

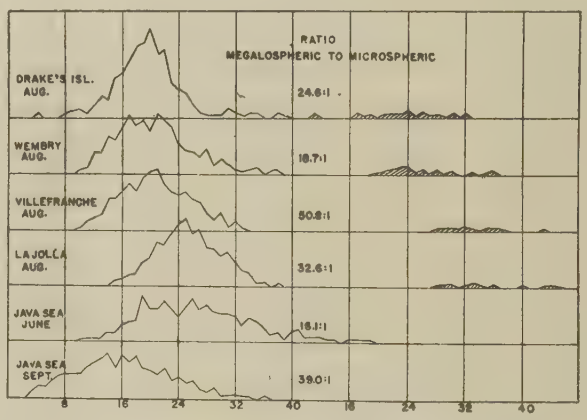

FIG. 6. Percentage frequency-distribution curves for growth stages in *E. crispum* based on the number of chambers in samples of populations numbering at least 500 individuals. The number of classes of growth stages represented suggests the length of the period of reproduction.

Wembry.—At Wembry, near the entrance to Plymouth Sound (fig. 1), the coralline algae upon which *E. crispum* lives are exposed to heavy surf produced by ground swells from the open channel. In the early spring the scouring action of the water delays the development of a substratum rich in the unicellular plants upon which the Foraminifera graze. A more limited supply of food delays the recovery of the Foraminifera from winter dormancy, reduces the rate of growth, and extends the periods of reproduction.

For these reasons the frequency-distribution curve for a sample taken at Wembry in August is broader, more depressed, and has more secondary modes than the curve discussed for a similar sample taken on the same day on Drake's Island within the more sheltered portion of the sound (fig. 6). Curves for samples taken on coralline algae at Cawsand and Bovisand were similar to those for samples taken at Wembry, because these stations also were exposed to heavy surf action. The mean number of chambers in the two samples represented by curves in figure 6 were 18.2 and 19.8, respectively—a difference of 1.6 chambers. At the usual rate of growth this is the equivalent of a difference of 10 days' to 2 weeks' growing time.

Villefranche.—The mean number of chambers in a sample of a population of *E. crispum* taken near Villefranche on the Mediterranean coast of France in August of the following year was 20.8 (fig. 6). The plankton content of the water in this region is known to be relatively low, and the season is considerably advanced compared with that at Plymouth. At Plymouth the spring of 1939 was more favorable to the Foraminifera and the organisms upon which they depend for food than was the preceding year, and conditions at Villefranche were probably likewise more favorable. Therefore the slight difference in the number of chambers produced suggests that the rate of growth at Villefranche is relatively low in spite of the fact that the tidal range in this region is so slight that the Foraminifera remain submerged at all times and are therefore not subjected to those adverse conditions which develop on a beach exposed at ebb tide.

The depressed nature of the curve for this sample is indicative of a period of reproduction that possibly was less intense than that which had resulted in the curve obtained for the Drake's Island sample.

La Jolla, California.—The curve for a sample taken from a tide pool near La Jolla, California, in August of 1937 (fig. 6) is less abrupt than that for the Drake's Island sample and more abrupt than those for samples from the other stations discussed. This suggests a spring outburst of phytoplankton production somewhat intermediate between those occurring at the other stations. The difference in the mean number of chambers produced before September at La Jolla could have resulted from a difference in the rate of growth of the geographical subspecies found locally, compared with that of the European subspecies, or from a more abundant and persistent supply of food.

The maximum number of chambers in tests of *E. crispum* from a sample taken at La Jolla in November at the end of the growing season was also comparably greater than the numbers obtained at the other stations. This difference in the limit of growth is probably due to a longer growing season and to more intense summer outbursts of phytoplankton production. This production results from the upwelling of water rich in nutrient salts from the intermediate depths—a movement brought about by the drift of surface water away from the coast in this region. This increased supply of plant food supports a luxuriant growth of algae along this coast, as seen in the great beds of the giant kelp, *Macrocystis pyrifera*. For reports of phytoplankton production at La Jolla, see W. E. Allen (1928).

Java Sea.—In the western portion of the Java Sea temperature, salinity, illumination, and other conditions remain relatively constant, and there is no evidence of a change of season on the islands of the region (fig. 7). In a shallow tropical sea nutrient salts are utilized by the microflora almost as soon as they become available, and an impoverished condition of the water as regards plant food and plankton organisms is presumed to persist at all times. Two samples of populations of Foraminifera from reef weed taken on Poelo Kelapa in the Thousand Islands near Sunda Strait show wider variations in growth stages than the conditions would suggest as possible (fig. 6). The relatively smooth depressed curves representing these populations show more classes of growth stages than the curves previously discussed, and when interpreted in the light of earlier observations, they suggest unusually prolonged periods of asexual reproduction and shorter periods of relative dormancy.

The absence of early juvenile stages in June (fig. 6) makes it obvious that no asexual reproduction had taken place for some time. The low ratio of megalospheric to microspheric individuals compared with the ratio obtained in September, and the occurrence of numbers of large megalospheric individuals, make it equally obvious that sexual reproduction was taking place, as was confirmed by the presence of gametes in many of the larger tests. The rate of growth, as determined from a series of samples taken at intervals of one month, was found to be somewhat in excess of that observed at Plymouth during the spring and early summer of 1939. Therefore the period of

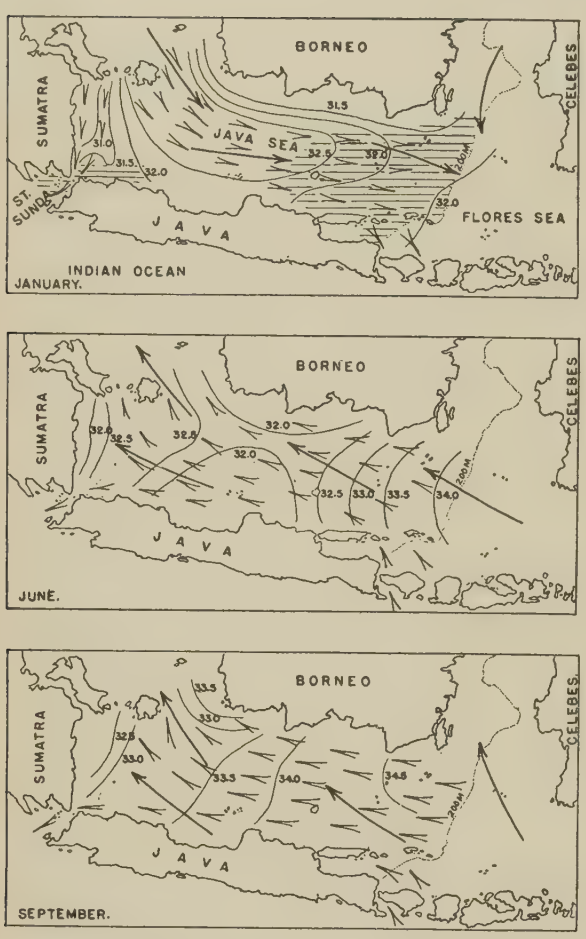

FIG. 7. Probable course of isohalines in the Java Sea. Darts indicate currents, and arrows wind direction. Water temperatures range from 26.5° C. to 28.0° C. with less than 0.5° C. difference between the surface and the bottom. The season of the West Monsoon extends from December to April, during which water from the shallow South China Sea enters the Java Sea, while the season of the East Monsoon lasts from April to December, during which typically oceanic water enters the Java Sea from the Flores Sea and Indian Ocean; thus the water of the Java Sea is exchanged with that of adjacent bodies of water twice each year. Surface water flows outward through Sunda Strait at all times, but there is evidence that a deep current enters the Java Sea through this strait from the Indian Ocean during the season of the West Monsoon at least in occasional years. From seasonal variations in growth stages in the Foraminifera and other evidence it would seem that this was the case in 1940. Shaded areas suggest regions in which species of *Globigerina* are most numerous in the bottom sediments, and where the largest numbers of certain fishes are taken. Conditions in December are similar to those in June except for the direction of the wind and currents.

asexual reproduction that had resulted in this generation of megalospheric gamonts had begun some four or five months earlier, probably in January at the height of the West Monsoon season, which extends from December to March.

Evidence of a second period of asexual reproduction is to be found in the September sample in the number of juveniles that had been recently produced; and from the absence of appreciable numbers of larger individuals it is evident that sexual reproduction had taken place some weeks earlier. This is further suggested by the high ratio of megalospheric to microspheric individuals and by the absence of cytological evidence of gametogenesis. The largest individuals in the June sample were probably produced during the period of reproduction that had preceded the one responsible for the major portion of this population.

From these deductions it would seem that the sequence of events in the life cycle of *E. crispum* is similar in all regions; that is, a period of comparative dormancy is followed by a brief period of growth which in turn is followed by sexual reproduction of megalospheric gamonts and shortly thereafter by asexual reproduction by microspheric agamonts. In the higher latitudes the period of winter dormancy probably results more from a shortage of food than from low temperatures, and in the spring, growth and reproduction are induced by the abundance of food that becomes available at the time of the spring outburst of phytoplankton production. From our experience with cultures we know that after a foraminifer has approached a more or less definite growth limit, a moderate supply of food is sufficient to induce reproduction; therefore it is logical to conclude that in the Java Sea an outburst of phytoplankton production or even a moderate supply of food organisms would induce reproduction in Foraminifera that were approaching the growth limit for the species.

No quantitative studies of phytoplankton production have been made in the Java Sea. Until this is done, it will be impossible to prove or disprove the occurrence of an outburst of phytoplankton production in the region of the Thousand Islands that would explain the occurrence of distinct periods of reproduction in these Protozoa. However, from the data available it would seem that such an outburst is altogether possible, as will be explained. The occurrence of such an outburst of phytoplankton production would also

contribute to a better understanding of the migrations of fishes reported by Hardenberg (1937).

We know that currents induced by the East Monsoon winds pass through the narrow channels between the Thousand Islands from April to December, and that this water, coming as it does from the shallow basin of the Java Sea, is impoverished in nutrient salts. Toward the end of the season, reproduction by the microflora probably approaches a minimum, and a shortage of food would reduce the activities of the Foraminifera to a state verging on dormancy.

Surface water is thought to flow outwards through Sunda Strait at all times, but from salinity determinations (Berlage, 1927) it is evident that in at least occasional years a deep stream of water from the Indian Ocean passes through the strait into the region of the Thousand Islands from December to April. From our observations on the distribution of the tests of pelagic Foraminifera in the sediments (Myers, 1942*b*), it is evident that this water continues along the north coast of Java as far as the Bay of Batavia, which also approximates the limit of the range of the fishes referred to, which enter this portion of the Java Sea only during the season of the West Monsoons. The deeper water of the Indian Ocean is richer in nutrient salts and mixes with the water of the Java Sea in the vicinity of the Thousand Islands. The enrichment of the water in this region would no doubt result in an outburst of phytoplankton production, and with a sudden increase in the supply of food organisms following a period of sparsity, the more mature Foraminifera reproduce. This is the only way in which we can account for the generation of *E. crispum* produced in January of 1940, which are represented by the June sample. Because this outburst of phytoplankton production developed slowly, the period of reproduction in the Foraminifera was extended, and the number of classes of growth stages increased in comparison with those in samples from other regions. As the individuals produced in January approached the growth limit of the species, only a moderate supply of food would be necessary to induce the period of reproduction that began prior to September. It is therefore unnecessary to presume a second period of intense phytoplankton production to account for the second generation. A difference in the state of maturity of individuals, produced as a result of a retarded phytoplankton outburst in January, would result in a corresponding difference in the time when the progeny reached maturity and in turn reproduced. This explains the similarity in the shape of the curves for the two samples under discussion. From these and related data it is evident that in this portion of the Java Sea the usual life span of *E. crispum* is six months, and the life cycle is completed within one year, or in one-half the time required in the higher latitudes.

Evidence of distinct periods of reproduction in these Protozoa suggests that water rich in nutrient salts entered the Java Sea from the Indian Ocean through Sunda Strait during the West Monsoon season of 1940.

Influence of Ecological Factors on Success of *Elphidium crispum*

The substratum.—*E. crispum* is most successful when submerged at all times, although it does occur on matted growths of algae at levels on the beach that are exposed for brief intervals on unusually low tides. They are most numerous in tide pools where the coralline algae remain relatively free from masking growths of filamentous algae, and where the scouring action of the surf does not remove these and other Foraminifera that remain free and active in the adult stage. *E. crispum*, with its smooth lens-shaped test and weakly developed pseudopodia, is well adapted to graze upon the microflora living upon the densely growing, semirigid fronds of *Corallina*. It is also found in lesser numbers on larger algae and eelgrass, but is more easily washed from this substratum by currents than a species in which at least the ventral surface of the test is flattened or in which the test becomes attached, as in *Planorbulina* and *Cibicides*.

In the sublittoral zone *E. crispum* is most numerous on a bottom composed of firmly packed sand or of a mixture of coarse sand and shell gravel. Suitable areas are usually overlaid with clear non-estuarian water and are o ten in the backwash of a tidal eddy, where, during much of the summer, shifting masses of larger algae that have broken away from the shore cover the bottom. Water-borne food and the rich microflora living on such a bottom contribute to the production of a maximum population of *E. crispum*.

On a sandy bottom the biconvex test of this species offers little resistance to currents, and the radiating pseudopodia loosely bind together a mass of sand which tends to prevent the foraminifer from being dislodged during periods of moderate turbulence. *E. crispum* is not always

numerous in areas similar to those described, but a reasonable amount of exploration along almost any coast will usually reveal a satisfactory source of material.

In protected bays, or in seas where winds of gale velocity are unusual, as in the Java Sea, mud bottoms often support a population of larger species of this genus, but where the empty tests are relatively uncommon in the sediments, it is well to presume that these had not originated in the immediate vicinity, especially if the depth of the water exceeds that at which photosynthetic organisms thrive.

It is somewhat difficult to understand why Foraminifera with vastly different types of tests should occur in the same population, or why species having a particular type of test should succeed under seemingly unfavorable conditions. However, the nature of the substratum definitely limits the distribution of many species, and one quickly learns where to look for a particular species, especially in coastal areas, although it is not always possible to explain why one area appears more favorable than another. In a later paper we shall discuss more fully the test morphology of the Foraminifera of the Java Sea in relation to the substratum upon which they live (see discussion).

Tides.—At Plymouth the amplitude for spring tides is from −7.3 feet to +8.2 feet, or 15.5 feet, while that for neap tides is from −3.8 feet to +3.8 feet, or 7.6 feet, and the extreme amplitude for the year is 19.1 feet. In this region there are two high and two low tides each day that are nearly equal, and the beach at the three-quarter-tide level, where *E. crispum* is most numerous, is exposed on spring tides for more than 7 hours, twice in each 24-hour period. Therefore tide pools at the three-quarter-tide level contain only surface water of minimum salinity and maximum temperature for many hours each day; further, the water entering these pools usually has been grazed upon by myriads of animals living at either higher or lower levels on the beach, so that, after the tide has receded below the level of the pool, the already impoverished food content of the water is further depleted by the animals living within the pool. The possible influence of food on the growth of *E. crispum* living under these conditions has been discussed; also the fact that this species is unable to survive more than mild dessication and therefore does not thrive on that portion of the beach exposed at low tide.

Since the ratio of microspheric to megalospheric individuals in tide pools is considerably higher than in the sublittoral zone (that is, 1 to 25 compared with 1 to 40 or even 50), it is evident that the sexual phase of the life cycle is more successful in tide pools. This is probably because there are no currents to wash away the gametes that are released after the tide has receded below the level of the pools (figs. 5 and 6). This assures an opportunity for a maximum number of pairs of gametes to unite in fertilization and for the zygotic amoebulae to become attached before the return of the tide and the resulting turbulent surf.

Abrupt changes in ecological conditions within a tide pool as a result of the tidal flux often make necessary rapid readjustments on the part of the inhabitants, particularly in the higher latitudes, as we shall endeavour to show. These ecological conditions, including the impoverished food content of the water, are responsible for the fact that in all latitudes populations of Foraminifera living in the intertidal zone have few species in common with populations living below low-tide level. In the following lists *Elphidium crispum* is the only species common to both life zones that exceeded one percent of the living Foraminifera in the samples studied.

TEN SPECIES OF FORAMINIFERA MOST NUMEROUS IN THE SUBLITTORAL ZONE NORTH OF DRAKE'S ISLAND IN AUGUST

	Percent
Elphidium crispum (Linnaeus)	28.0
Rotalia beccarii (Linnaeus)	22.0
Elphidium striato-punctatum (Fichtel and Moll)	16.2
Verneuilina polystropha Reuss	9.5
Nonion depressula (Walker and Jacob)	8.4
Quinqueloculina lata Terquem	5.3
Textularia conica D'Orbigny	3.8
Bulimina elegans D'Orbigny	1.1
Pyrgo depressa D'Orbigny	.6
Quinqueloculina seminula (Linnaeus)	.4
Total	95.3

EMPTY TESTS OF SPECIES OCCURRING IN THE SAMPLES THAT WERE NOT PRESENT IN THE LOCAL POPULATIONS, COMPARED WITH THE NUMBERS OF LIVING FORAMINIFERA IN THE SAMPLE

	Percent
Cibicides refulgens Montfort	54.2
Planorbulina mediterranensis D'Orbigny	15.1
Discorbis globularis D'Orbigny	12.9

TEN SPECIES OF FORAMINIFERA MOST NUMEROUS IN TIDE POOLS OF THE LITTORAL ZONE IN AUGUST

	Percent
Discorbis globularis D'Orbigny	66.3
Elphidium crispum (Linnaeus)	15.2
Hemidiscus perversus Sidebottom	5.0
Cibicides refulgens Montfort	2.1
Cibicides lobulatus (Walker and Jacob)	1.6
Planorbulina mediterranensis D'Orbigny	1.3
Discorbis rosacea (D'Orbigny)	1.3
Quinqueloculina lamarckiana D'Orbigny	1.0
Quinqueloculina contorta D'Orbigny	.6
Gromia oviformis Dujardin	.6
Total	95.0

Temperature.—*E. crispum* and its geographical subspecies succeed where the mean surface temperatures in the sea for the three summer months range from 7° C., as in sheltered bays in south Greenland and the Falkland Islands, to 30° C. in the Red Sea and the Java Sea (Brady, 1884). In Plymouth Sound the mean water temperature for February is the lowest for the year, with an average for 8 years of 8° C., and August is the warmest month, with an average of 16° C., while the total range for the 12 months is 8° C. August is also the month of maximum temperature fluctuations between the surface and the bottom as well as within a single tidal phase, although neither of these fluctuations at Plymouth exceeds 2° C. On a sunny day the temperature of the surface water in a tide pool may be 3° C. above that in Plymouth Sound, although at the depth at which the Foraminifera are most numerous the temperature difference is usually less than 1° C. Therefore the maximum change in temperature to which Foraminifera in a tide pool would be subjected between two tidal phases would seldom exceed 4° C. However, this is equal to about one-half the mean annual range of temperature in the latitude of Plymouth. Under similar conditions in other regions fluctuations of 10° C. may take place within a matter of minutes on successive days with little or no apparent effect upon the distribution of either the Foraminifera or the algae upon which they live.

Growth through the addition of new chambers to the tests of individuals that had survived the winter was first observed in February, when the mean surface temperature was little more than 8° C. or slightly above the minimum for the year. Both growth and sexual reproduction approached a maximum soon after March 15, when the mean temperature was less than 0.5° C. above the minimum for the year. On the other hand, winter dormancy in these Foraminifera, as determined from the amount of shrinkage in the protoplasm, was well advanced by October 15, when the mean temperature was nearly 14° C., or 6° C. above that of March, when the rate of growth and reproduction was at the maximum for the year. In the littoral zone asexual reproduction was well advanced by April 15, when the mean temperature was 9.5° C., while in the sublittoral zone the same conditions were not reached until May 1, when the deeper and somewhat colder water of this life zone was about the same temperature as that observed in tide pools two weeks earlier. This is the only instance in which there would seem to be a correlation between temperature and the life activities of the species under consideration. However, the second period of asexual reproduction in the sublittoral zone occurred in late September, when the mean surface temperature was 15° C., or only about 1° C. below the maximum for the year, and the difference in temperature between the first and second periods of asexual reproduction in the tide pools was 6.5° C., which is equal to a difference of three-fourths the annual range for temperatures in the sound.

When the temperature in cultures of other species of Foraminifera was reduced, growth and reproduction were discontinued (Myers, 1935). Should someone succeed in culturing *E. crispum*, a state verging on dormancy might be induced by lowering the temperature. Such a laboratory experiment would be interesting, but would have little significance because it would not explain why the temperature in the sea at Plymouth was far lower during the period of greatest growth and reproduction in this species than it was during the first half of the period of winter dormancy. Temperature is no doubt an important factor in that it tends to limit the rate of metabolic activities in an organism, but we are forced to conclude that in the vicinity of Plymouth Sound temperature has little influence upon the life activities of *E. crispum*, although it probably does limit the activities of certain other species.

Salinity tolerance.—The maximum range of salinity within a tidal cycle for organisms living near low-tide level on Drake's Island in Plymouth Sound was 26.6 ‰[1] and 34.9 ‰ at low and high tides, respectively, which is a difference of 8.3 ‰ or a change of 5.8 atmospheres of osmotic pres-

[1] ‰ indicates parts per thousand.

sure in a little more than 10 hours. In March, the month of greatest growth and reproductive activities in *E. crispum*, the salinity range for the complete tidal cycle may be only 25.3 ‰ to 26.4 ‰ at low and high tides, respectively, although surface salinities as low as 19.5 ‰ do occur. This low-salinity surface water may remain in tide pools for as long as 7 hours on a spring low tide (Milne et al., 1938). Therefore it is evident that fluctuations in salinity within Plymouth Sound are not sufficient to be a critical factor in the distribution of *E. crispum*. Otherwise this species would not have occurred at all levels on the beach below the three-quarter-tide level.

A heavy rain may cause from 2 to 4 inches of fresh water to collect at the surface of a pool, but this does not mix appreciably with the denser sea water except where a pool is exposed to strong winds. Consequently this fresh water does not usually extend to the depths at which Foraminifera are most numerous.

The upper limits of salinity tolerance in a balanced aquarium that had been allowed to slowly evaporate was 48 ‰, which is far in excess of salinities observed in the ocean. Therefore a range in salinity from 19.5 ‰ to 48.0 ‰ is well within the salinity tolerance of this species. However, continued low salinity appears to be unfavorable, for *E. crispum* does not thrive in estuaries.

Nutrient salts.—*E. crispum*, in common with most free-living rhizopods, is holozoic. It is thus doubtful whether the dissolved nutrient substances in the sea have an appreciable effect upon its metabolism except in so far as they influence the production of the photosynthetic organisms which constitute the principal source of food for this foraminifer. Periods of growth and reproduction in *E. crispum* more or less coincide with the periods when nitrates, phosphates, and silicates are most abundant. However, they also coincide with the periods when food organisms which utilize these nutrient salts are also most abundant (fig. 2). In the winter, when the nutrient salt content of the water approaches a maximum, the limited amount of illumination reduces the productivity of photosynthetic organisms to a minimum; and with a reduction in the amount of particulate food, the Foraminifera reach an inactive state verging on dormancy. The shape of the test of this species is altered by the rate of growth, but the thickness of the walls seems to bear no relationship to the mineral content of the water or to other chemical and physical conditions in the water at any latitude.

Hydrogen ion or pH.—The hydrogen ion concentration of sea water in equilibrium with the air lies close to pH 8.1. In the sea it may vary from 7.8 to 8.3 owing to the withdrawal of carbon dioxide by plants during photosynthesis. This tends to increase the alkalinity or the pH, while carbon dioxide exhaled by animals or derived from the oxidation of organic matter tends to increase the acidity or decrease the pH. The buffer reaction of certain salts in the sea tends to maintain a state of equilibrium between the hydrogen, bicarbonate, carbonate, and hydroxyl ions, but in a tide pool an unbalanced condition may result from the predominance of plant life over animals. On a sunny day alkalinities as high as 9.5 have been observed (Harvey, 1939), while in estuaries, such as that of the River Tamar entering Plymouth Sound, the amount of carbon dioxide released by the decomposition of organic matter may increase the acidity to 7.5 (Huntsman, 1918).

Small species of Foraminifera survive a pH of 7.0 that develops when excessive amounts of organic matter in an aquarium decompose, although *E. crispum* usually falls from the walls when the pH is depressed below 7.3. Where other ecological conditions are favorable, the pH is rarely a critical factor in the success of benthonic Foraminifera.

Oxygen tension.—An increase in temperature decreases the solubility of oxygen in sea water. Oxygen derived from the atmosphere, supplemented by that released in the synthesis of carbohydrates by the phytoplankton, approximately saturates the surface water in the sea. In tide pools a sunny day often causes the release of more oxygen by the macroflora than the water can dissolve, with the result that the excess oxygen collects in small bubbles on all surfaces. Zoöxanthellae living as inclusions in the cytoplasm of the Foraminifera provide a direct source of oxygen, but these symbiotic algae do not occur at the latitude of Plymouth, and are not essential to the success of most species (plate 1, fig. 5). With an increase in depth, there is usually a decrease in the oxygen content of the water, but in the shallow water of Plymouth Sound and the English Channel, there is sufficient vertical circulation to maintain a high oxygen tension at all seasons (Harvey, 1934). For these reasons oxygen tension is seldom if ever a critical factor.

Illumination.—Most Foraminifera are positively phototropic and negatively geotropic. Prior to reproduction these responses cause the Foraminifera to take up positions on the algae or other objects where the gametes or progeny, when released, are easily dislodged and disseminated by currents. In the late fall this tropistic behavior is evidently reversed, particularly in the microspheric generation, and we find the Foraminifera retreating into crevices between the holdfasts and encrusting fronds of coralline algae or into other protected niches. This reaction probably explains the gradual increase in the proportion of microspheric to megalospheric individuals observed throughout the winter, which could not have resulted from sexual reproduction at this time because there was no cytological evidence of gametogenesis by megalospheric gamonts and no microspheric juveniles were observed (fig. 5 and Myers, 1942*a*). The absence of zoöxanthellae in the Foraminifera at Plymouth may be attributed to the long winters with few hours of sunlight each day.

Turbulence and currents.—Turbulence and current action are the principal forces responsible for the dispersal and geographical distribution of the Foraminifera, although floating algae and other material, as well as wandering or migrating animals that become encrusted with a fauna and flora consisting of algae, barnacles, hydroids, bryozoans, and other organisms, are contributing factors. The gametes of the Foraminifera, as in other animals, are a transition stage that remains viable for only a short time. It is thus probable that the principal method of dispersal is through the juvenile or even adult individuals that are thrown into suspension by turbulence and transported to new areas by currents (Myers, 1936).

When surface sediments are thrown into suspension by wind-induced turbulence, the Foraminifera, because of their lesser density and shape, settle through the water more slowly than quartz sand and come to rest at or near the surface of the heavier sediments. When Foraminifera are buried to a depth not exceeding 5 to 7 times the diameter of the test in sandy sediments, or in the case of the larger *E. crispum* to a depth of about 1 cm., they are able to escape. If buried more deeply, they die within a few months, or even more quickly should the oxygen supply beneath the surface of the sediments be insufficient to sustain life—a condition often made evident by the blue-black sulphides deposited on the walls of the tests or by the presence of iron pyrites within the tests. Should the Foraminifera survive, later periods of turbulence may deposit them sufficiently near the surface so that they regain it and resume normal activities. Most Foraminifera taken between October and March, both in tide pools and in the sublittoral zone, remained inactive when placed in an aquarium, and it is apparent that in the sea they would make little or no attempt to remain at the surface of the sediments during the winter. For this reason it was necessary to examine approximately 500 times more surface sediments in December than in August, to obtain suitable samples of populations of *E. crispum*. From this we may conclude that within the zone of turbulence few Foraminifera remain at the surface of the sediments during the winter, and the rate of survival is suggested by the difference in the numbers of living Foraminifera per unit area before and after the period of frequent and heavy storms. In one area the population in September, as determined from material obtained through the use of a special suction grab (Hunt, 1926) that takes a sample having a definite unit area, was 1,225 per square foot, compared with 245 per square foot in March. From these and other data it would seem that turbulence and current action in the sublittoral zone are responsible for the destruction of at least 80 percent of all individuals that survive beyond the juvenile stage. In tide pools from which most of the larger algae are stripped by the surf after October, a similar proportion of the population is also destroyed by these same agencies (Myers, 1942*a*).

From these observations we may conclude that in some areas turbulence and current action are important factors in the success or failure of a species, and this probably explains the absence of appreciable numbers of *E. crispum* in samples taken in the English Channel.

Turbidity.—Turbidity in the sea limits the depth to which light penetrates, and in turn the depth of the column of water in which photosynthetic organisms are able to utilize nutrient salts. Therefore it limits the production of the food of the Foraminifera both in the phytoplankton and in the microflora living on the bottom as well as the larger algae that provide a habitat essential to the success of many species of Foraminifera. The effect of these conditions upon the distribution of the Foraminifera was studied in our survey of the Java Sea. Certain species that were numerous at depths of 10 to 20 meters in the eastern half of the sea were found at increasing

depths down to 80 meters as the entrance to the Flores Sea was approached (fig. 7). Secchi disc readings at the limits of this range vary from 5 to 12 meters and from 25 to 30 meters, respectively. The depth to which photosynthetic organisms thrive is considered to be about 2½ times the Secchi disc readings.

The River Plym discharges quantities of clay into Plymouth Sound during heavy rains. *E. crispum* living on larger algae survives where this clay remains in suspension for days or even weeks at a time, and where this material is occasionally silted out in such quantities as to cover the algae with a heavy film. However, it would be unusual to find appreciable numbers of Foraminifera living on mud bottoms adjacent to such an area.

From our knowledge of the distribution of benthonic Foraminifera based upon the protoplasmic casts that remain after the sample is treated with acid, and from our knowledge of the food requirements of these Protozoa as determined from cytoplasmic inclusions, it would seem that the depth of the photic zone as influenced by turbidity has a decided effect upon the quantity of food available, the nature of the substratum, and the depth to which many species of Foraminifera are able to succeed.

Animal Associates

The densely growing, semirigid fronds of coralline algae provide a protected habitat for myriads of small animals, including many species of Foraminifera. A 1-cc. sample of the finer washings from a handful of these algae, taken from a tide pool on Drake's Island in July, contained 573 living Foraminifera, including 91 *E. crispum* in addition to 474 animal associates. These animal associates included representatives of most of the phyla of the animal kingdom and consisted of minute species and juvenile stages of larger species. Among the phyla most abundantly represented, there were 204 Mollusca, 118 Annelida, 115 Arthropoda, 16 Bryozoa, and 13 Nematoda.

A sandy bottom in the sublittoral zone supports a smaller population of animal associates because of the smaller numbers of sessile mollusks and the larger size of the majority of the inhabitants. No numerical study of populations of animal associates was made from this area, although the suction grab used for population studies of the Foraminifera was actually devised for this purpose.

Animal associates not only compete with the Foraminifera for space, but many are more or less dependent upon the same food organisms. We have determined that certain of these associates, including annelids, arthropods, and flatworms, consume numbers of smaller Foraminifera and the juvenile stages of larger species; and that, in turn, these associates provide the Foraminifera with a limited amount of food in the form of eggs, less violently active larval stages such as veligers and trochophores, and the residual protoplasm contained in cast exoskeletons and the bodies of dead organisms.

Parasites and Enemies

From 3 to 5 percent of *E. crispum* taken in the sublittoral zone in the early spring contain developmental stages of the protozoan parasite *Trophosphaera planorbulinae* (Calvez), which Calvez (1936) has assigned to the order Coccidiida. This parasite develops in the protoplasm of the larger chambers and may extend throughout the entire test, destroying the host. Nematode worms also invade the tests in the early spring, and although paraffin sections usually show the protoplasm shrunken away from these worms, the presence of eggs and larval stages in the tests suggests that this is actually a case of parasitism and not a mere invasion of the tests by the worms.

The common shrimp, *Crago vulgaris*, is for the most part a bottom-feeding herbivore, but during the winter, when the microflora on the bottom is sparse, the digestive tract of this arthropod often contains, in addition to sand and plant detritus, from 8 to 14 or more entire or broken Foraminifera that were living at the time they were ingested. It is interesting that *C. vulgaris* continued to feed upon the Foraminifera for some weeks after the microflora that is the natural food of this animal had again become abundant. When food is sparse, most animals living in the sea eat whatever nutrient material is available, and animals feeding upon a substratum composed of minute plants and animals ingest Foraminifera living in this substratum either by accident or through choice. This explains the occurrence of Foraminifera in the digestive tract of many invertebrates, including echinoderms, annelids, flatworms, and arthropods. Certain holothurians and astropectins living in the Tropics ingest enormous numbers of Foraminif-

era, and at times these no doubt constitute the principal source of food. The tests of Foraminifera found in the faeces of many invertebrates show no more evidence of erosion than the tests found in the sediments, and it is evident that the tests of most species could pass through the digestive tract of the invertebrates studied many times before they would be appreciably dissolved.

Discussion

No previous attempt to determine the possible influence of ecological conditions in the sea upon the productivity of the Foraminifera has come to our attention. The present work is therefore limited in scope, but the results and conclusions demonstrate that the methods used could, if applied to other species living under various conditions, contribute to a better understanding of problems in stratigraphy and paleoecology where the fossilized tests of Foraminifera are the principal biotic constituent in geological marine sediments.

Many factors contribute to limit the geographical and bathymetric distribution of a species. Temperature or conditions associated with temperature are of primary importance. Depth and salinity have also been stressed as limiting factors, but the Foraminifera are animal organisms and as such depend upon particulate food for sustenance; therefore the nature of the food available to these Protozoa is largely determined by ecological conditions, including the depth of the water.

The Foraminifera are perhaps as diverse in their food requirements as in the way in which they utilize it; that is, the food may be ingested before being digested, as in many of the unattached Milliolidae, or it may be reduced by digestive ferments in the pseudopodia before it is taken into the test, as in species of *Elphidium* and *Calcarina*, where the apertures of the test are minute. Some species depend primarily upon organisms in the plankton, as in the pelagic Globigerinidae, or upon sessile forms, as in the Homotremidae, while others depend upon organisms in the substratum, as in most free-living Rotaliidae and Miliolidae, or they may utilize organisms found both in the substratum and in the plankton, as in species of *Elphidium* and *Amphistegina*. In the Foraminifera, as in other free-living Protozoa, growth and reproduction take place when the supply of food is in excess of the basic requirements of the animal. In *E. crispum* the varying length and width of the chambers suggest interruptions in the rate of growth which result from a shortage of food or other adverse conditions. When the rate of growth, as determined from frequency-distribution curves based on growth stages, is correlated with measurable ecological conditions in the sea, it is possible to evaluate to some extent the relative importance of these conditions as limiting factors in the success of the species. These varying ecological conditions, acting simultaneously upon the several phases of the life cycle, make it somewhat difficult to estimate the possible effect of a particular condition when comparable changes in magnitude are taking place in other conditions. In Plymouth Sound it would seem that conditions which favor the production of an abundant microflora both in the water and on the bottom also favor growth and reproduction in the Foraminifera. Knowledge of the limits of variation in ecological conditions in this area, where the species is most successful, eliminates from serious consideration as limiting factors similar conditions in other regions, unless it can be proved that these conditions are detrimental when reinforced by other conditions. A hypothetical example would be the possible adverse effect of temperatures similar to those observed in the Java Sea (27° C. ± 1° C.) and of a salinity approaching the minimum observed at Plymouth (25 ‰). We know that *E. crispum* succeeds in both regions and that these conditions acting independently are not detrimental, but together they might limit the distribution of the species. Unfortunately data on this and similar problems are not yet available.

The nature of the substratum, particularly where there is a considerable macroflora, and the degree to which the bottom sediments are disturbed by turbulence and currents are also of primary importance. We find that there is often a distinct correlation between test morphology and the nature of the substratum upon which a species lives. *Marginopora*, *Planorbulina*, and similar attached forms are protected from surf and tidal currents because the large discoidal tests are exceedingly thin. The test of *Operculina* is equally large and depressed, but this form is limited to soft mud bottoms. The equally large *Heterostegina* and *Operculinella* have tests in which the umbilical region has considerable thickness, and these species, provided with heavier tests, are restricted to firmer bottoms where the sediments contain much coarse material or are covered with

a scattered macroflora. Species such as *Tinoporus* and *Calcarina*, in which the test is provided with heavy perforated spines, are found on densely growing algae, while *Rotalia pulchella*, with proportionately longer spines, is often the dominant species where the surface sediments are little more than a colloidal suspension. However, it is also true that certain species inhabit bottoms to which they seem poorly adapted. For example, it is difficult to understand why species with massive tests and not unusually long or numerous pseudopodia, such as *Elphidium craticulata* and *Rotalia papillosa*, should inhabit soft mud bottoms. These observations will be more fully discussed in a later paper.

Elphidium crispum or its geographical subspecies are usually numerous in clear, non-estuarian coastal waters where an appreciable macroflora exists on the bottom. Either this or related species are often found on mud bottoms at depths which, in our experience, do not greatly exceed that of the photic zone and where the bottom sediments are not disturbed by wind-induced turbulence or strong tidal currents during the major portion of the year. The present bathymetric distribution of *E. crispum* and the relative abundance of the tests in certain Tertiary formations make this species a useful guide to the probable depth and the proximity to the coast at which the sediments were deposited.

Summary

1. The life activities of the foraminifer *Elphidium crispum* (Linnaeus) were studied from frequency-distribution curves of growth stages, based on the number of chambers in the test of samples of populations taken monthly for 12 months. The observed sequence of events was correlated with measurable ecological conditions in the sea.

2. The major part of the work was based on samples taken in the vicinity of Plymouth, England, where extensive studies have been made on marine ecology. Additional samples from the Mediterranean coast of France, the coast of southern California, and the western portion of the Java Sea are also discussed in relation to conditions prevailing in the respective areas.

3. It was found that in temperate regions the life span is usually one year, and in tide pools the life cycle, including a sexual and an asexual phase, is completed in two years, while below low-tide level three or even four years may be required. In tropical waters the life span is about six months, and the life cycle is completed in one year.

4. Growth and reproduction are limited for the most part to the spring months, when water temperatures are low, and the period of winter dormancy is well advanced before the temperature has fallen appreciably below the summer high.

5. The major source of food consists of unicellular plants living on the bottom and in the water. Therefore growth and reproduction in this protozoan closely parallel the phytoplankton cycle in the sea.

6. Individuals of *E. crispum* living below low-tide level produced 40 percent more chambers and a 60 percent larger test than those living in tide pools, largely as a result of the relative abundance of food. The rate of growth has a decided effect upon test morphology in this species and varies somewhat from year to year.

7. Variations in salinity, temperature, illumination, nutrient salts, turbidity, and other conditions seem to have little influence upon the productivity of the species, as compared with food.

8. Approximately 80 percent of the individuals produced in these two life zones are destroyed in the late fall and winter as a result of turbulence and current action.

9. Populations in excess of 1,000 individuals per square foot were observed. The annual rate of increase over the winter low is about thirty-fold and forty-fold in the littoral and sublittoral zones, respectively, and the rate of mortality is equal to the rate of productivity.

10. Differences in ecological conditions account for the fact that the littoral and sublittoral zones have few species of Foraminifera in common, although the tests of both populations are found in offshore sediments.

11. Knowledge of the influence of food upon the life activities of the Foraminifera in regions where quantitative studies of phytoplankton production have been made, makes it possible to deduce much from similar frequency-distribution curves of growth stages in populations from regions where no studies of the food content of the water have been made, as in the Java Sea.

12. Animal associates, parasites, and enemies are discussed, as well as the influence of test morphology upon the feeding habits and the distribution of other species.

Acknowledgments

In a series of papers extending over the past 12 years investigators have stressed the need for further work in the field of marine ecology in relation to the Foraminifera, and have explained the value of this work in stratigraphy and paleoecology. Recognition of this need is responsible in part for the recent erection of a subcommittee of the National Research Council to deal with these problems. The present paper is the second of a series designed to extend our knowledge of the significance of the tests of Foraminifera found in recent and geological marine sediments. The field work was made possible through grants from the Guggenheim Memorial Foundation and the Treub Laboratories of the Netherlands East Indies, and is being continued on a Penrose Grant from the Geological Society of America at the Woods Hole Oceanographic Institution. The major portion of the field and technical work was done at the Laboratory of the Marine Biological Association at Plymouth, England, where extensive studies have been made on chemical and physical conditions in the sea in relation to plankton production. My wife Ethel is responsible for much of the technical work and assisted with the manuscript.

Note.—Since this paper was submitted for publication, Dr. Margret W. Jepps has published an account of her studies on *Elphidium crispum* made at Plymouth, England, in 1938–39, the same year that the major portion of the material for the present paper was collected. The two papers have little in common other than the further substantiation of the basic facts of the life cycle established by Lister (1895), who also worked at Plymouth. Neither of these recent studies has advanced materially our knowledge of the origin and fate of the nuclei in the several phases of the life cycle, but it is to be hoped that through the establishment of the time and duration of the periods of reproduction it will be possible to solve this fundamental problem, for through its solution the chromidial theory of Hertwig as applied to the Foraminifera will no longer be tenable.

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PLATE I

FOOD OF THE FORAMINIFERA

FIG. 1. Paraffin section of a 56-chambered megalospheric *Elphidium crispum* showing the arrangement of the chambers and the position of the single nucleus.

FIG. 2. Section through a *Quinqueloculina* taken in a tide pool that had fed upon fragments of larger algae, filamentous algae, diatoms, and other unicellular plants.

FIG. 3. Section of a *Discorbis* reared in a culture that had fed upon vast numbers of diatoms. The earlier formed chambers and the nucleus show in the upper half of the figure.

FIG. 4. Nucleus of a megalospheric *E. crispum* at a higher magnification than figure 1.

FIG. 5. Section through several chambers of a *Peneroplis* taken on a dead coral reef in which the zoöxanthellae and food organisms probably exceeded in volume the amount of protoplasm belonging to the foraminifer.

FIG. 6. Dividing nuclei, and diatom frustules contained in the cytoplasm of a *Patellina* produced in a culture.

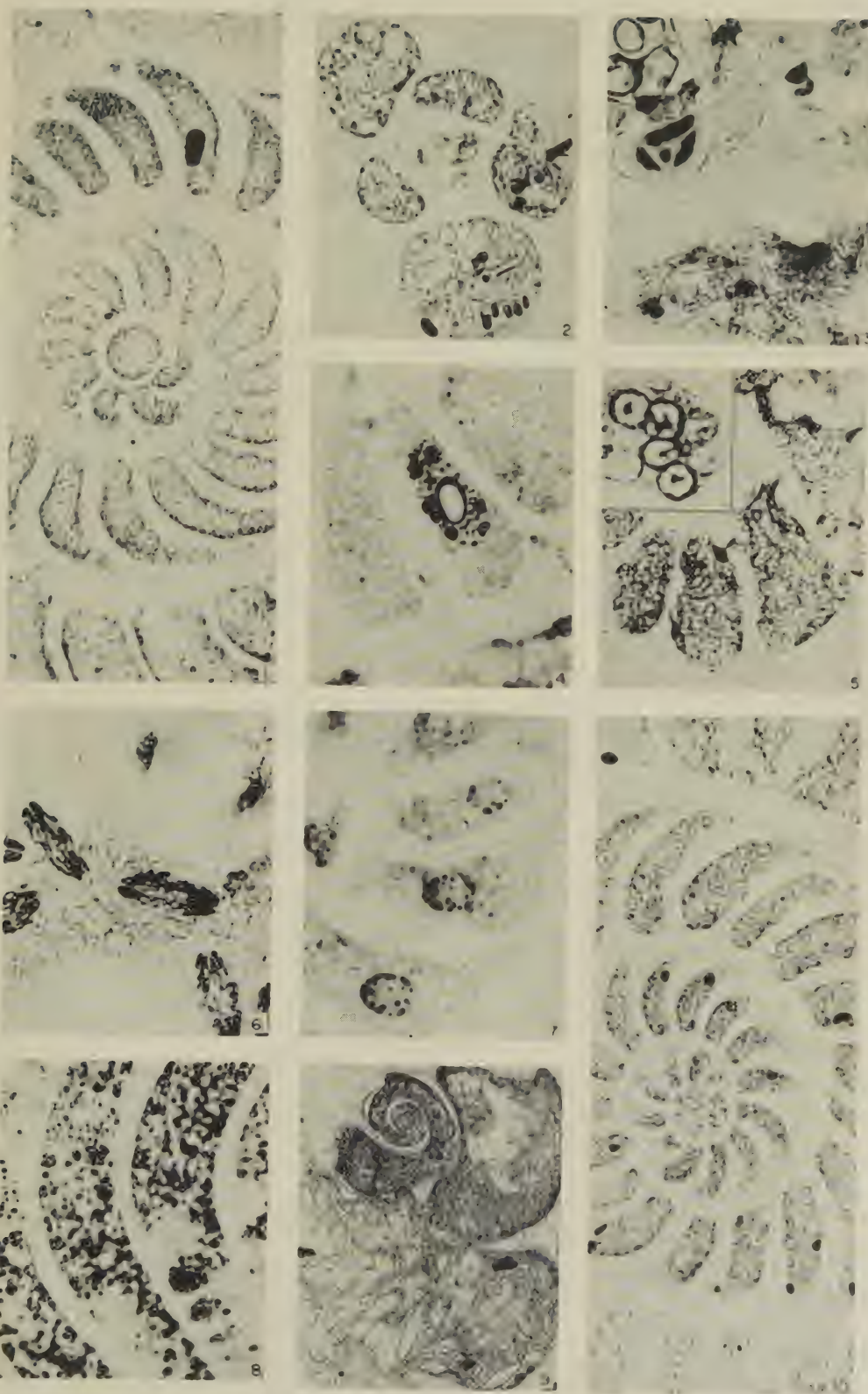
FIG. 7. Several nuclei of a microspheric *E. crispum* at the same magnification as figure 4.

FIG. 8. Partially digested flagellated organisms, presumably the gametes of larger algae contained in the distal chambers of *E. crispum* taken with a dredge. Similar material is contained in the sections of the larger chambers shown in figure 10.

FIG. 9. Section of *Articulina* taken on reef weed showing vast numbers of diatom frustules. The large nucleus is contained in another section.

FIG. 10. Section through a 64-chambered *E. crispum* showing many nuclei and the arrangement of the chambers. Compare with figure 1.

PLATE I



(For description, see opposite page.)

Contribution No. 328

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THE DISTRIBUTION AND REPRODUCTION OF SAGITTA ELEGANS ON GEORGES BANK IN RELATION TO THE HYDROGRAPHICAL CONDITIONS

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(Biological Laboratories, Harvard University and Woods Hole Oceanographic Institution) [1]

During the past few years an investigation has been undertaken of the factors underlying the productivity of Georges Bank, an extensive fishing area lying east of Cape Cod (Fig. 1). The economy of this area depends upon various interdependencies of the fish populations, the bottom fauna, and the plankton; and all are profoundly affected by the complex of strong currents and persistent eddies which are found on the Bank. In order to understand the essential ecological relationships, it is therefore necessary to gain a knowledge of both the hydrography and the biology of the waters of the region.

The present study of the abundance, distribution, and seasonal cycle of reproduction of the chaetognath, *Sagitta elegans*, was undertaken first because this species forms a prominent element in the zooplankton of Georges Bank, and second because Sagitta may be used as a "current indicator" to aid in unravelling the involved current system of the region. This species is a relatively large, easily recognized member of the zooplankton and its body length and maturity stage are readily determined. The life span of *Sagitta elegans* is sufficient to bridge periods of six weeks or more, with the result that in cases where observations are repeated each month, the same population may be identified from one cruise to the next. This condition presents a desirable contrast to more rapidly reproducing organisms, such as diatoms, in which populations of large dimensions may appear or disappear within a week or so.

Certain relatively recent investigations of the ecology of Sagitta in other regions are available for comparison, but none had the advantage of our quantitative collection method, nor the opportunity for revisiting as frequently over a two-year period an extensive net-work of stations as characterized the present undertaking. The breeding and growth of *Sagitta elegans* was studied by Russell (1932; 1933) off Plymouth, England, and by Pierce (1941) in parts of the Irish Sea. *Sagitta elegans* has been employed successfully as a current indicator in British waters by Russell (1939) and the distribution of the species by currents in the Gulf of Maine has been critically investigated by Redfield and Beale (1940).

Collection and Analysis of Material

Samples of plankton and hydrographic data for the present study were obtained from the research vessel, "Atlantis", during eleven cruises to Georges Bank from September 1939 to June 1941 (Table I). On each cruise a net-work of 21 to 52 stations was occupied over the Bank. In all cruises (except that of

[1] Contribution No. 328.

January, 1940) the stations were ordinarily placed at 15-mile intervals on five or six parallel sections, about 25 miles apart, running SE and NW across the Bank and into the immediately adjacent waters. The location of the stations is indicated in the charts showing the distribution of Sagitta (Figs. 5 and 6). The stations covered the region from South Channel on the southwest to the eastern tip of Georges Bank and from the deep basin of the Gulf of Maine on the northwest to the edge of the continental shelf on the southeast. No stations

TABLE I

List of cruises to Georges Bank

Cruise no.	Date	No. of stations	Station serial nos.
89	Sept. 6–13, 1939	52	3629–3680
93	Jan. 4–11, 1940	21	3726–3746
95	Mar. 21–Apr. 2, 1940	35	3792–3826
96	Apr. 17–27, 1940	26	3827–3852
97	May 9–16, 1940	33	3856–3888
98	June 1–8, 1940	36	3892–3927
100	June 19–27, 1940	36	3932–3967
112	Mar. 21–Apr. 2, 1941	33	4177–4209
113	Apr. 15–23, 1941	34	4210–4243
114	May 7–14, 1941	34	4244–4277
116	May 28–June 4, 1941	33	4278–4311

could be made in the immediate vicinity of Cultivator and Georges Shoals. The segment of the ocean covered by the station net-work of each cruise was more than 150 miles long and 100 miles wide, or an area larger than the states of Massachusetts, Connecticut, and Rhode Island combined (Fig. 1).

Standard hydrographic observations for salinity and temperature were made at every station and Secchi disc measurements of transparency were carried out during daylight stations. Studies of certain chemical characteristics of the water and of the phytoplankton population were undertaken by collaborating investigators (Sears, 1941; and Riley, 1941 and 1942).

The zooplankton was collected at each station by means of two or more hauls with Plankton Samplers (Clarke and Bumpus, 1940) and one haul with a stramin net. Sagittae were taken in adequate numbers in both types of equipment and the two sets of hauls served as a check on one another.

The opening of the Plankton Sampler, which is 12.7 cm. in diameter, is provided with a shutter, and each instrument contains a meter which records the amount of water filtered by the net. In the present case, the instruments were equipped with No. 2 silk nets (22 strands/cm.) and "oblique" hauls [1] were made at a speed of about 2 knots for periods of 25 to 40 minutes. Ordinarily between 10 and 20 cubic meters of water were filtered during each tow, but the action of

[1] In an "oblique" haul the net is towed horizontally but is raised in steps so that the whole depth of the stratum concerned is sampled. The Sampler could be towed safely down to within three meters of the actual bottom.

the tide or of clogging was such that values as low as 5 m.^{3} and higher than 30 m.^{3} were recorded. This variation makes clear the need for measuring the amount of water which actually passes through the net. The Samplers were arranged vertically so as to divide the total depth of water into two or three strata and, when feasible, were attached to the same cable. The uppermost

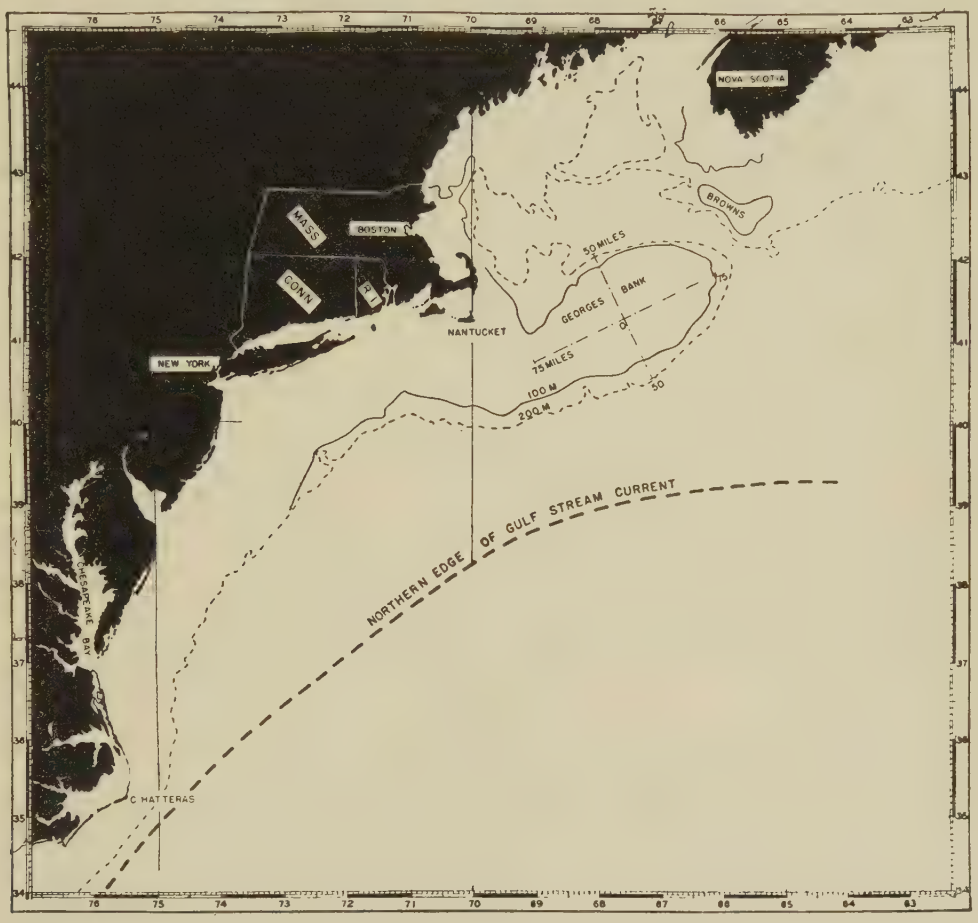

FIGURE 1. Orientation map of Atlantic Coast. The location and relative size of Georges Bank are indicated.

instrument sampled the "Shallow" Stratum, extending from a depth of 25 m. to the surface. The lower limit of this stratum corresponded roughly with the position of the thermocline in those areas where it existed. At stations where the water was less than 75 m. deep, the "Second-depth" Stratum extended from the bottom to 25 m. In water deeper than 75 m., however, the remaining distance to the bottom (or to a maximum depth of 200 m.) was divided into two equal parts and these comprised the "Second-depth" and the "Deep" Strata

respectively. The vertical distribution of the sagittae could therefore be studied on the basis of these strata:

Stratum	Water less than 75 m.	Water more than 75 m.
"Shallow"	0 m. to 25 m.	0 m. to 25 m.
"Second-depth"	25 m. to bottom	25 m. to half distance to bottom (or to half distance to 200 m.)
"Deep"	——	Remaining distance to bottom (or to 200 m.)

The stramin net (Diameter: 1.5 m., Mesh: 6 strands/cm.) was equipped with rollers at the lower edge of its frame in order that it could be safely lowered until it touched the bottom. One "oblique" haul was made from the bottom (or from a depth of 200 m.) to the surface at each station. When proper allowance

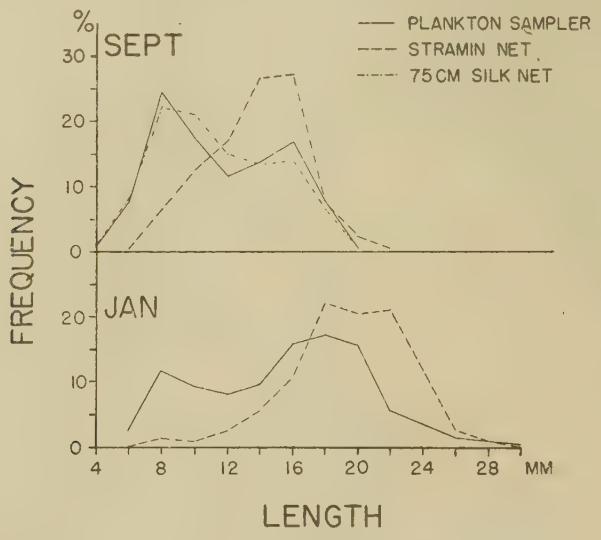

FIGURE 2. Comparison of length frequency distribution of *Sagitta elegans* for the following types of nets:

Plankton Sampler (12.7 cm. in diameter) with No. 2 silk.
Silk net (75 cm. in diameter) with No. 2 Silk.
Stramin net (1.5 m. in diameter).

was made for the difference in the sizes of the apertures of the stramin net and the Plankton Samplers, a good agreement was found between the numbers of sagittae taken by the former and the sum of the catches of the latter at each station.

Before the work was begun, it was doubted whether the relatively small Plankton Sampler would catch the larger sizes of an active animal, such as Sagitta, in their true proportions. For the first cruise (September 1939), therefore, a silk net 75 cm. in diameter and of the same mesh, was towed immediately below the Plankton Sampler. Since comparison of the length frequency distribution of the sagittae taken by the two sizes of nets showed exceptionally good agreement (Fig. 2), it is felt that the catch of the Plankton Sampler can be relied upon. In the January cruise sagittae as large as 30 mm. in length were retained

by the Sampler. On the other hand, the stramin net was shown not to retain adequately the smallest sizes of sagittae. For these reasons and especially because of the accuracy of the determinations of depth and volume with the Plankton Samplers, the ensuing analysis of the abundance and distribution of the Sagitta populations is based primarily upon the hauls with these instruments. The present observations can therefore be placed on a quantitative basis not hitherto possible.

The sagittae were separated from the remaining plankton in the laboratory and the species present were identified and enumerated.[1] The great majority of sagittae were *Sagitta elegans* but specimens of *S. serratodentata* and a smaller number of *S. enflata* were encountered in certain hauls from the periphery of Georges Bank. For each station the average number of *S. elegans per cubic meter* was calculated *for each stratum* by dividing the number caught in each haul by the volume of water filtered by the Plankton Sampler. The total number of individuals *under each square meter* of sea surface was found by multiplying the number per cubic meter for each stratum by the thickness of the appropriate stratum and then adding these products together. Finally the *average number of animals per cubic meter for the whole water column* at each station was obtained by dividing the foregoing value by the total depth of water at each station. These average values per cubic meter (or per ten cubic meters) have been plotted on the charts showing quantitative distribution (Figs. 5, 6, and 10), but they may readily be re-converted to the "per square meter" basis by multiplying by the depth in each case.

Length measurements were made of all specimens of *Sagitta elegans* in each haul up to a maximum of 50. The stage of maturity was also determined for the individuals of this species in most of the hauls in each cruise from all parts of the Bank. Animals from each haul (usually between 20 and 50 individuals) were stained by the method described by Pierce (1941, p. 115), and then were classified as Stage I—*Immature*, Stage II—*Intermediate*, or Stage III—*Mature*, following the criteria of Russell (1932, p. 134).

General Hydrographical Conditions

The depth of the major portion of Georges Bank lies between 40 m. and 100 m., although areas of less than 25 m. occur in the north central portion, and the Shoals themselves are covered by only 5 to 15 m. of water. Along the northern edge of the Bank the bottom drops rapidly from about 40 m. to more than 200 m. as the deep basin of the Gulf of Maine is approached. Along the southern edge the depth changes somewhat more gradually from 100 m. to 200 m. Beyond 200 m. it increases rapidly to about 2000 m.

Georges Bank is therefore, roughly speaking, a submerged, flat-topped plateau (Fig. 3), and it presents a sufficiently large obstacle to water movement to produce a profound effect on the ocean currents of this region. Although the details of the water movements over and around the Bank have never been adequately determined, especially for the colder part of the year, it has been well established in general that during the summer months at least, water from the Gulf of Maine

[1] The authors are indebted to Miss Dorcas Delabarre for technical assistance in the analysis of the Sagitta material.

does not flow directly across the Bank but tends to move around the eastern and southern margins of the Bank in a clockwise direction, leaving a relatively stationary eddy of water over the central part of the Bank. From the point of view of the ecology of the Bank, our interest in the current system lies in the question of the degree of permanence of this eddy, and in the extent to which the "bank water" can be regarded as biologically isolated from the surrounding regions.

The eddy on the Bank might be dislodged by relatively slight changes in the strength or position of the surrounding ocean currents (Iselin, 1939), or it might be disrupted by the action of certain local agents. The strong tidal currents on

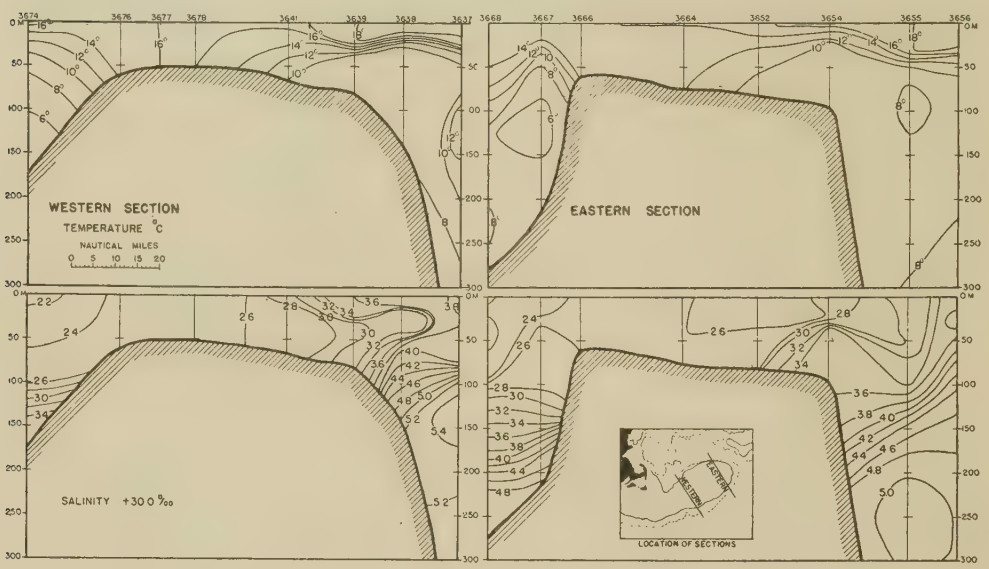

FIGURE 3. Vertical sections for temperature and salinity for September 1939 (cruise 89). The contour of Georges Bank is indicated by the cross hatching from the Gulf of Maine on the left to the edge of the Continental Shelf on the right. Station numbers appear at the top of the diagrams. The figures on the salinity curves are to be increased by 30 to give the actual values in parts per mille.

the Bank cause the overlying water to oscillate in generally elliptical paths, the long diameters of which may exceed eight miles. Winds, which frequently reach gale velocities, sweep unimpeded across the area, tending to force the surface water along with them. The danger would thus appear to exist that from time to time the bank water might be swept entirely off the Bank, carrying with it the pelagic stages of animals which, as adults, could live only in a bank environment, or removing an element of the plankton which is essential to the economy of the Bank. Even though no cataclysmic dislocation of the bank water occurred, it is important to know to what extent a dilution or a renewal of the water mass may take place through continuous or intermittent admixtures of new water from one direction or another.

The turbulence produced by the tidal currents and by the wind in the relatively shallow water overlying Georges Bank causes a vertical mixing of the

water which results in a nearly uniform distribution of temperature and salinity from top to bottom at all seasons of the year, particularly in the central part of the Bank. The bank water thus contrasts sharply with the surrounding water masses, which are typically stratified during all except the winter months. Since the temperatures and salinity values on the Bank are generally intermediate between those of the surface and the deeper strata on the Gulf of Maine but usually much lower than those of the water lying to the south, we know that the bank water is originally derived, in a large part at least, from the Gulf (Figs. 3 and 4). That portion of the Bank over which vertically uniform water was found is termed the *Mixed Area*, and all stations at which the salinity does not vary by more than 0.2 part per mille from surface to bottom are considered to

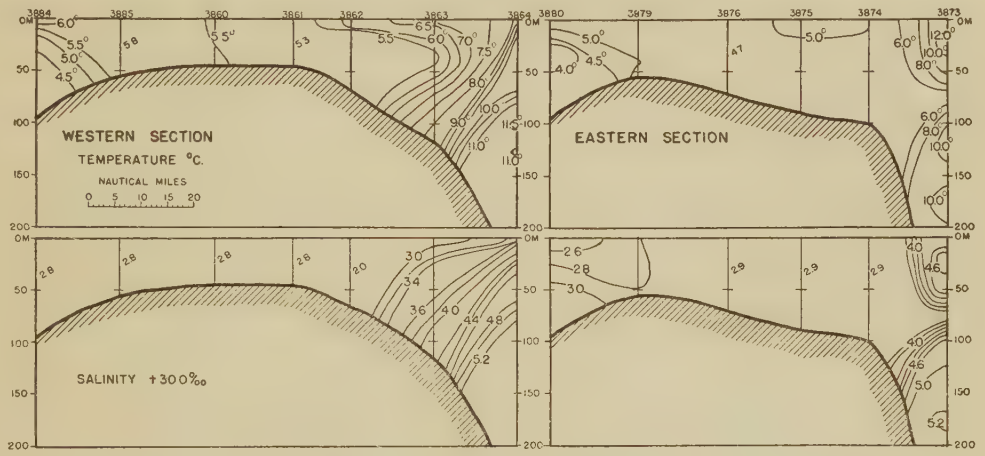

FIGURE 4. Vertical sections for temperature and salinity for May 1940 (cruise 97).

lie within it.[1] The limits of the Mixed Area are ordinarily rather sharp, and have been indicated by a heavy broken line in the charts of Sagitta distribution for each cruise (Figs. 5, 6, and 10).

The vertical uniformity of the temperature and salinity within the Mixed Area presents an ecological condition for the Bank organisms which is quite unlike that for the oceanic forms living in the stratified water of the adjacent deeper areas. Moreover, seasonal changes in these factors are somewhat damped by the continued vertical mixing, as is seen by reference to Tables II and III. The seasonal range in temperature for the Mixed Area extended from a minimum of about 2.5° C. to a maximum of over 16° C. Surface temperatures in the regions to the north and to the south of Georges Bank were generally higher in summer and lower in winter. Furthermore, during the winter pelagic animals living at the surface in these neighboring areas could find warmer water by descending to lower strata, and similarly during the summer they could escape excessively high temperatures by seeking greater depths. In contrast, the fauna

[1] Uniformity of salinity, rather than temperature, was taken as the criterion for the Mixed Area because in the present situation salinity is less easily modified after the water has reached the Bank.

TABLE II

Comparison of temperatures in the Mixed Area and in the Stratified Water at the margins of Georges Bank

Values given are for typical stations near the center of the Bank and in the deeper water to the north and to the south. At the stations in the Mixed Area temperatures did not vary by more than 1.6° C. from surface to bottom and in most cases the variation was much less.

Date	Mixed Area	North Margin		South Margin	
	Aver. temp.	Surface	100 m.	Surface	100 m.
Sept., 1939	16.3° C.	16.4° C.	6.3° C.	19.3° C.	9.3° C.*
Jan., 1940	4.1	3.3	6.8	4.6	6.2*
Mar., 1940	2.5	2.1	5.3	2.8	3.9*
Apr., 1940	3.7	3.5	3.4	4.7	11.2
May, 1940	4.8	5.3	4.1*	6.1	7.8*
June 1–8, 1940	7.2	10.4	3.0*	10.8	9.3
June 19–27, 1940	8.5	10.6	3.1	10.3	10.2
Mar., 1941	2.7	3.3	4.1	2.9	4.9
Apr., 1941	3.9	4.3	4.5	3.2	4.9
May, 1941	4.6	6.5	5.4	4.0	4.5
June, 1941	6.8	9.1	3.6	7.3	5.4

* Value at somewhat less than 100 m.

TABLE III

Comparison of salinities in the Mixed Area and in the Stratified Water at the margins of Georges Bank

Values given are for typical stations near the center of the Bank and in the deeper water to the north and to the south. At the stations in the Mixed Area salinities did not vary by more than 0.2‰ from surface to bottom.

Date	Mixed Area	North Margin		South Margin	
	Aver. salinity	Surface	100 m.	Surface	100 m.
Sept., 1939	32.5‰	32.1‰	32.5‰	33.4‰	33.8‰*
Jan., 1940	32.8	31.5	33.7	32.9	33.2*
Mar., 1940	32.8	32.4	33.2	32.7	33.0*
Apr., 1940	33.0	32.5	33.1	33.0	35.3
May, 1940	32.8	32.5	33.2*	32.7	34.1*
June 1–8, 1940	32.8	31.8	32.9*	33.1	33.5*
June 19–27, 1940	32.7	32.0	32.9	32.5	34.4
Mar., 1941	32.7	32.8	33.1	32.7	33.4
Apr., 1941	32.5	32.5	33.2	32.1	33.5
May, 1941	32.5	32.3	33.4	32.1	33.4
June, 1941	32.7	32.3	32.8	32.6	33.5

* Value at somewhat less than 100 m.

of the central bank waters could reach a materially different temperature only by migrating laterally entirely out of the Mixed Area. A similar situation obtains in regard to salinity, although there is little evidence that changes in salinity, *per se*, of the magnitude encountered in this region are of ecological importance. On the other hand, differences in density, which result in large part from the salinity, are bound to be critical for passively floating organisms, and the lack of a pronounced vertical density gradient in the Mixed Area, as well as the excessive turbulence there, presents a very special problem for such forms.

Quantitative Distribution of Sagitta Elegans

Vertical Distribution

The numerical abundance of *Sagitta elegans* varied greatly among individual hauls, ranging from a maximum of 165 specimens per cubic meter [1] to zero. When the hauls of each cruise are considered together, however, certain general trends in the changes in the Sagitta population become clear. The changes in horizontal distribution from cruise to cruise will be presented in the next section. In this section the variations in the vertical distribution will be considered as derived at each station from the separate hauls with the closing Plankton Samplers for the Shallow Stratum, the Second-depth Stratum, and the Deep Stratum. At stations where the depth of water was less than 75 m. the Second-depth haul extended to the bottom. Since this situation obtained at the majority of stations within the Mixed Area, the chief comparison for vertical distribution is between the Shallow Stratum and the Second-depth Stratum.

It is obvious that vertical distribution at stations made during the day (between the hours of sunrise and sunset) had to be distinguished from the situation at stations made during the night, since a diurnal migration of the animals was to be expected (Russell, 1933). Furthermore, if a vertical migration of the Sagitta tended to take place, very different conditions would be met with according to whether the station was in the Mixed Area or in the Stratified Area. If the animals encountered a thermocline, their movement might be stopped, or reversed (cf. Clarke, 1934). For the foregoing reasons the hauls upon which the analysis of the vertical distribution of *Sagitta elegans* is based, have been subdivided into those made at stations in the Mixed Area and those made at stations in the Stratified Area and have been further subdivided in each case into day and night hauls.

The *average* abundance of *Sagitta elegans* for all stations in each of these categories varied considerably from cruise to cruise (Table IV). In September the larger number of animals was found in the Second-depth Stratum in all cases, although at night in the Mixed Area an almost equally great number was taken at the upper level. In the winter and early spring of 1940 much smaller average numbers of sagittae were encountered and the differences in the various strata were not large. A tendency for the largest hauls to occur in the Deep Stratum is to be noted for May 1940, but this generality does not hold for the more sizable catches of the June cruises of that year. During the early spring of 1941 small numbers of sagittae were again encountered and their vertical distri-

[1] Shallow stratum haul, May 30, 1941, made in south central part of Bank and consisting of very small individuals.

TABLE IV

Numerical abundance of S. elegans *in the separate strata. Average number per cubic meter in the indicated categories.*

X indicates an abundance of less than 0.1/m³. Values placed in parentheses are based on a total of less than 5 hauls.

Month	Cruise	Stratum	Mixed Area		Stratified Area	
			Day	Night	Day	Night
Sept., 1939	89	Shallow	5.3	17.5	0.7	1.6
		2nd Depth	10.8	17.9	8.1	(8.5)
Jan., 1940	93	Shallow	2.4	6.8	——	0
		2nd Depth	3.8	7.6	——	X
		Deep	(0.5)	(0)	——	0
Mar., 1940	95	Shallow	2.4	1.8	0	X
		2nd Depth	2.4	1.1	0	0.1
		Deep	(1.9)	(0.2)	X	X
Apr., 1940	96	Shallow	1.8	2.2	X	(0)
		2nd Depth	3.6	1.4	0.2	(0)
		Deep	(2.8)	——	(0.3)	(0)
May, 1940	97	Shallow	2.5	5.1	0.2	0.6
		2nd Depth	6.2	5.1	0.2	0.3
		Deep	(9.0)	(1.0)	16.6	13.0
June 1–8, 1940	98	Shallow	16.9	3.3	4.5	(3.3)
		2nd Depth	40.4	50.1	3.8	(5.9)
		Deep	(13.1)	(25.0)	3.6	(8.7)
June 19–27, 1940	100	Shallow	35.6	20.8	5.3	17.4
		2nd Depth	58.6	44.5	1.8	9.5
		Deep	——	(58.4)	3.4	12.6
Mar., 1941	112	Shallow	2.4	2.2	0	0.8
		2nd Depth	2.5	2.8	0	0.8
		Deep	(1.1)	(1.2)	X	1.2
Apr., 1941	113	Shallow	2.1	0.8	0	0.4
		2nd Depth	3.2	1.9	0	0.9
		Deep	(1.3)	(0.1)	0.1	0.1
May, 1941	114	Shallow	1.4	0.6	0	0.3
		2nd Depth	3.5	0.6	X	X
		Deep	(0.6)	(0)	0.3	1.0
June, 1941	116	Shallow	46.9	14.6	2.5	0.7
		2nd Depth	45.6	17.8	1.4	1.9
		Deep	(3.4)	(45.8)	1.6	2.5
Averages		Shallow	10.9	6.9	1.3	2.3
		2nd Depth	16.4	13.7	1.6	2.5
		Deep	3.7	14.6	2.9	3.9

bution was found to be generally uniform. This situation also held for May of that year, but in June much larger catches were made especially in the two upper strata for the day hauls and for the Deep Stratum for the night hauls in the Mixed Area.

In order to ascertain what tendency existed toward vertical diurnal migration it is not satisfactory to employ the foregoing average values because of the likelihood that a few large hauls would obscure differences occurring in stations with smaller representation. Accordingly a calculation has been made of the percentage of stations in each category for each cruise at which the number of *Sagitta elegans* in the Second-depth haul was greater than in the Shallow haul (Table V). When the data are scrutinized on this basis, it becomes clear that a

TABLE V

Comparative vertical distribution of S. elegans *for day and night hauls*

Percentage of Stations at which the number of animals per cubic meter in the "Second-Depth" haul was greater than in the "Shallow" haul. Values placed in parentheses are based on a total of less than 5 cases.

Month	Cruise	Mixed Area		Stratified Area	
		Day	Night	Day	Night
Sept., 1939	89	100%	29%	93%	50%
Jan., 1940	93	60	57		(100)
Mar., 1940	95	64	43		(67)
Apr., 1940	96	71	25	(100)	
May, 1940	97	86	50	60	(0)
June 1–8, 1940	98	70	71	(67)	(67)
June 19–27, 1940	100	75	100	20	33
Mar., 1941	112	78	70		(50)
Apr., 1941	113	73	(75)		(100)
May, 1941	114	75	(50)	(100)	(100)
June, 1941	116	46	80	(25)	50
Averages		73%	59%	66%	62%

definite vertical migration was taking place in both the Mixed Area and the Stratified Area at the time of the September cruise, since the majority of the animals were found below 25 m. in the day time and above 25 m. at night. A similar tendency, but less marked, was encountered in the first four cruises of 1940 and in the May cruise of 1941 for the Mixed Area. A reversal of the situation is to be noted for the June cruises in both 1940 and 1941, for in those cases the Second-depth hauls were greater at night at more stations than during the day. Taking the average for all cruises it is apparent that in all situations the deeper hauls were numerically greater in more than 50% of the cases. However, the variations encountered in vertical distribution and in diurnal migration from cruise to cruise show that the reactions of Sagitta in maintaining its vertical position in the water change materially according to the season or in relation to size and stage of maturity. A similar conclusion was reached by Russell (1933).

Horizontal Distribution Throughout the Year

The quantitative aspects of the distribution of *Sagitta elegans* will be examined in relation to the location of the water masses on Georges Bank for September

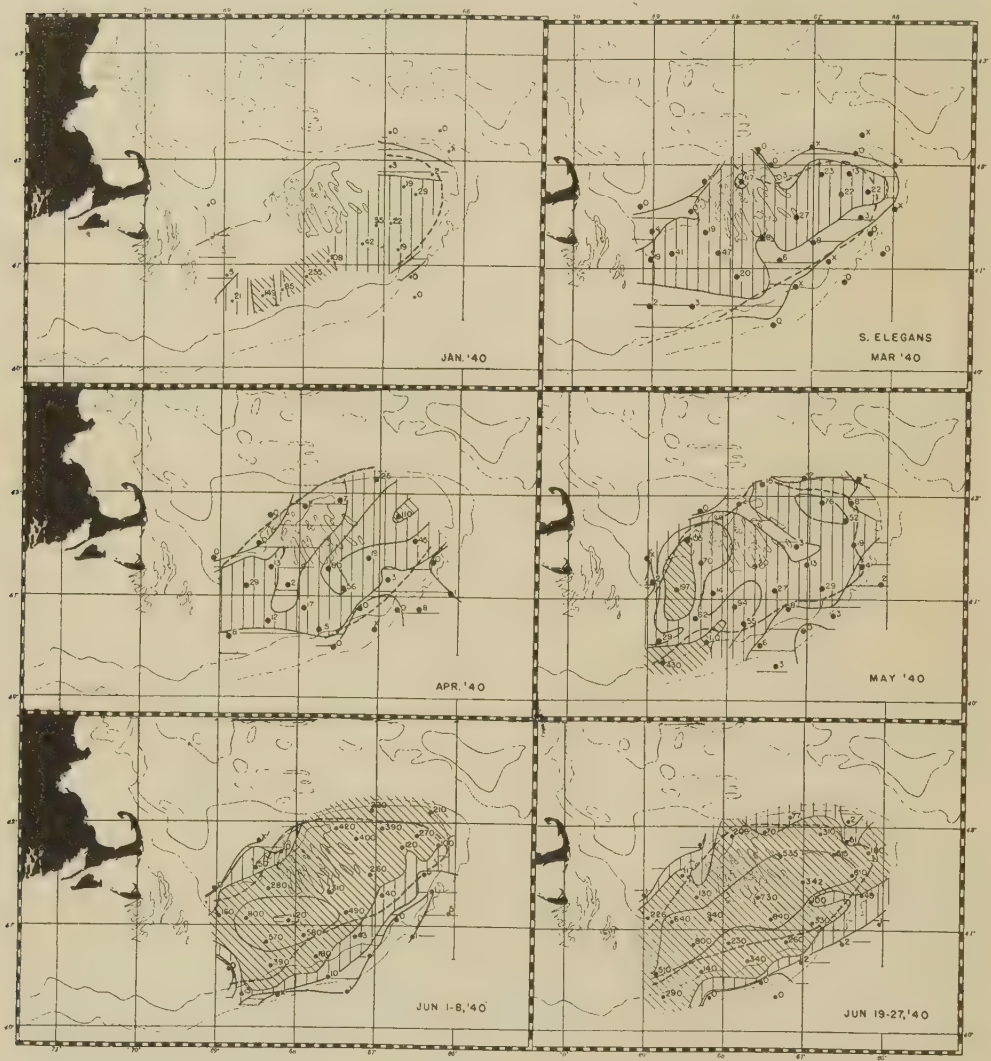

FIGURE 5. Distribution of *Sagitta elegans* on Georges Bank during 1940. January (cruise 93), March (cruise 95), April (cruise 96), May (cruise 97), June 1–8 (cruise 98), and June 19–27 (cruise 100). Average numbers per 10 cubic meters for whole water column for all stages. Plankton Sampler hauls. Boundary of Mixed Area indicated by heavy broken line.

1939 (Fig. 10), for the winter and spring of 1940 (Fig. 5), and for the spring of 1941 (Fig. 6). In each chart the station positions are designated by black dots and the *average* number of sagittae per 10 cubic meters *for the whole water column*

at each station is indicated.[1] Contour lines representing concentrations of 1, 10, (50), 100, (500) and 1000 individuals per cubic meter have been drawn in. Progressively dense cross-hatching indicates areas of increasing numerical abundance. In addition, the position of the margin of the vertically homogeneous water of the Mixed Area for each cruise has been indicated by a heavy broken line superimposed independently on each chart.

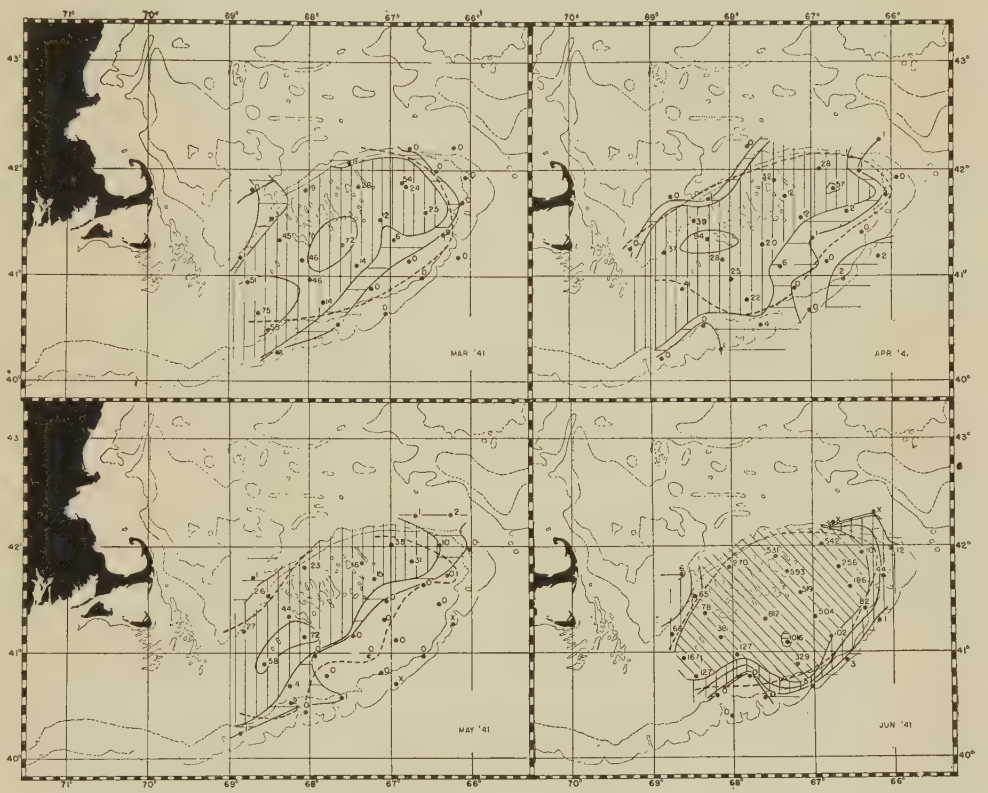

FIGURE 6. Distribution of *Sagitta elegans* on Georges Bank during 1941. March (cruise 112), April (cruise 113), May (cruise 114), and June (cruise 116). Average numbers per 10 cubic meters for whole water column for all stages. Plankton Sampler hauls. Boundary of Mixed Area indicated by heavy broken line.

Inspection of the chart for September 1939 (Figure 10, upper left) reveals the fact that at the time of this cruise extremely small numbers of *Sagitta elegans* occurred around the margins of the Bank (except possibly in the north central region). It is clear that the area of greatest concentration of this species lay in the center of the Bank. During this period the Mixed Area did not cover the whole of Georges Bank but was confined chiefly to the central portion except in the north where it extended beyond depths of 100 m. A rough correspondence

[1] The total number of sagittae *under each square meter* of sea surface may be derived from these values by multiplying by the depth (see "Collection and Analysis of Material").

is seen to exist for this cruise between the contour for 100 sagittae per 10 cubic meters (or 10 animals per cubic meter) and the limits of the Mixed Area.

A similar scrutiny of the distribution of *Sagitta elegans* may be undertaken for the succeeding cruises from Figures 5 and 6. A great variation in the numerical strength of this species during the year is indicated by the fact that although numbers greater than 10/m.^{3} were encountered at nine stations in the September cruise, only one to four stations were as rich during the next four cruises. However, in each of the June cruises of 1940 about 20 stations yielded an average abundance of more than 10/m.^{3} and one station of more than 100/m.^{3} Similarly in 1941 during the first three cruises there were no hauls containing more than 10/m.^{3} but in the June cruise this value was exceeded at 18 stations and there was one instance of an abundance greater than 100/m.^{3} Although it was unfortunately not possible to make observations in every month of the year, as would have been desirable, the available information strongly indicates that the numerical strength of *Sagitta elegans* in the Georges Bank region reaches a low ebb in the winter and early spring, and attains high values beginning in June and perhaps extending through the summer.

In each of the eleven cruises the center of abundance of *Sagitta elegans* was found to be located within the central portion of the Bank and numbers dropped off toward the margin. Along the southern edge of the Bank the concentration of this species dwindled to a very small proportion and frequently to zero, especially beyond the 200 m. contour. Similarly low numbers were usually encountered along the eastern and northern margins although in some cruises an insufficient number of stations was occupied beyond the Bank to make certain of the limits of the population to the north. Since in most cases *Sagitta elegans* occurred in abundance at the westernmost stations of each cruise, we have definite indication that, at certain seasons of the year at least, numbers of this species are transported by westerly currents across South Channel toward Nantucket Shoals.

When the center of abundance of *Sagitta elegans* is compared with the location of the Mixed Area, it is clear that in spite of the changes in position of the latter from cruise to cruise, the greatest concentrations of the species were always found within the Mixed Area, and a close agreement existed between the contours of abundance and the boundary line of the mixed water (Figs. 5, 6, and 10). In addition to the situation in September, 1939, already described, striking cases of conformity between the distribution of the *S. elegans* population and the extent of the Mixed Area are seen in May and June (Fig. 6). During the May cruise stratified water was found to occupy the southern portion of the top of the Bank extending for 30 miles or more from the southern edge toward the center, whereas in June the mixed water largely covered this region. Corresponding to this shift in the position of the water masses *S. elegans* was found to be almost completely absent from the southern half of the Bank in May, but in June its distribution extended to the southern edge of the Bank.

In general the abundance of *S. elegans* tended to be relatively uniform for all the stations within the Mixed Area during each cruise. This fact showed that the spacing of the stations in this area was sufficiently close. The uniformity was no doubt due in large measure to the turbulence of the water in the Mixed Area and would not necessarily be expected in other regions of uniform hydrographic conditions but with less water movement.

Sagitta elegans is therefore chiefly abundant within the Mixed Area of Georges Bank, and during the periods covered by the present cruises, at least, this species appears to be largely isolated from surrounding regions. Evidence has been presented above that a small part of the population may be carried to the west at certain seasons by the movement of water around Nantucket Shoals. It is unfortunate that our observations could not have been extended to Cape Cod and to the waters north of the Cape in order to ascertain whether the sagittae of Georges Bank ever attain any important relationship with populations occurring in that region. As far as our present data go, however, no significant connection is indicated between the concentration of *S. elegans* in the vicinity of Massachusetts Bay reported by Redfield and Beale (1940) and the population on the Bank.

Growth and Breeding of Sagitta Elegans

Seasonal Changes in Length

The specimens of *Sagitta elegans* taken during the present investigation varied greatly in size, covering a range in length from 4 mm. to 30 mm. The frequency distribution of the sizes for all the hauls of the 1939–1940 cruises may be examined from the histograms of Figure 7. It is seen that no specimens longer than 20 mm. were taken in September, but individuals as long as 30 mm. occurred in January, March, and May. The modal length increased from 16 mm. in September to 18–20 mm. in January, and to 24 mm. in March. In April the modal length was 22 mm. These larger sizes were also represented in May and June, but in diminishing numbers.

Specimens of *Sagitta elegans* as small as 6 mm. were present in the September cruise in numbers nearly as great as the intermediate sizes. In January the presence of a secondary mode at 8 mm. suggests the simultaneous existence of two generations. In March and April, however, the smaller sizes were reduced to extremely small numbers and the intermediate sizes were poorly represented. Smaller individuals appeared in May and were more abundant in that month than the larger categories. By early June the numerical strength of this new crop of small Sagitta had increased ten fold and by late June they were still abundant. The modal length increased from 6 mm. in May to 8 mm. in June. Although in the late June cruise there were many more Sagitta in the 4 mm. class, an even greater augmentation of the sizes larger than 10 mm. was observed. Similar changes in the relative abundance and length distribution of this species were encountered during the cruises of 1941.

Scrutiny of the length distributions at the various individual stations and within the different strata revealed in general no tendencies for segregation. In most cases both large and small specimens were represented in the same relative proportions at the various depths at each station. Although considerable variation in length frequency occurred from station to station, nevertheless in most cruises there was no consistent tendency for large or small individuals to appear in certain parts of the Bank. An exception to the foregoing statement occurred in the cruise of May 1940, as shown in Figure 8, in which individual frequency distributions have been plotted for each station with sufficient numbers. In this case it is seen that at the easternmost stations only large specimens of

Sagitta elegans were taken, whereas at the western stations the smaller sizes definitely predominated. Evidently the remnant of the older animals persisted chiefly in central and eastern eddies, while the production of younger individuals was beginning most actively in the western parts of the Bank (see below).

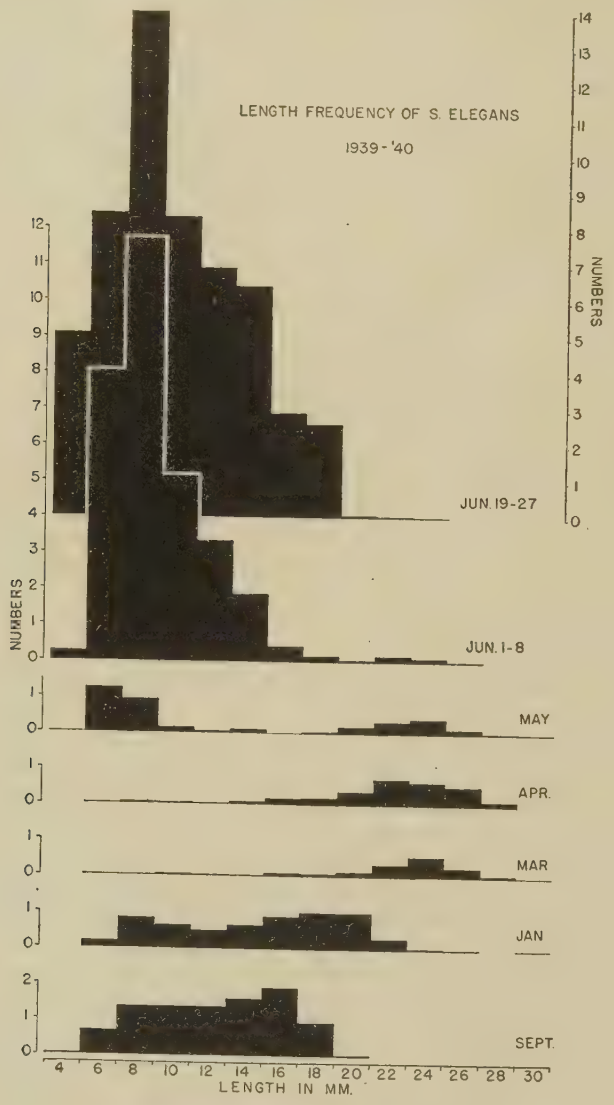

FIGURE 7. Average frequency distribution of lengths during 1939–40. Average numbers per cubic meter of *S. elegans* for each cruise.

Maturity Stages and Breeding Periods

Length measurements alone are not sufficient for determining the breeding periods of Sagitta because very great variations exist in the sizes of the three

stages of maturity. This fact is amply demonstrated by the graphs of Figure 9, in which the length frequency distribution of each maturity stage has been plotted on a percentage basis for each cruise. Here it is seen that immature specimens (Stage I) may attain a length of 16 mm. or more, but individuals as short as 8 mm. may definitely have attained the "intermediate" condition (Stage II). Furthermore some specimens grew to lengths of 24 mm. or more while still in Stage II, while other individuals became completely mature (Stage III) at a length of 12 mm.

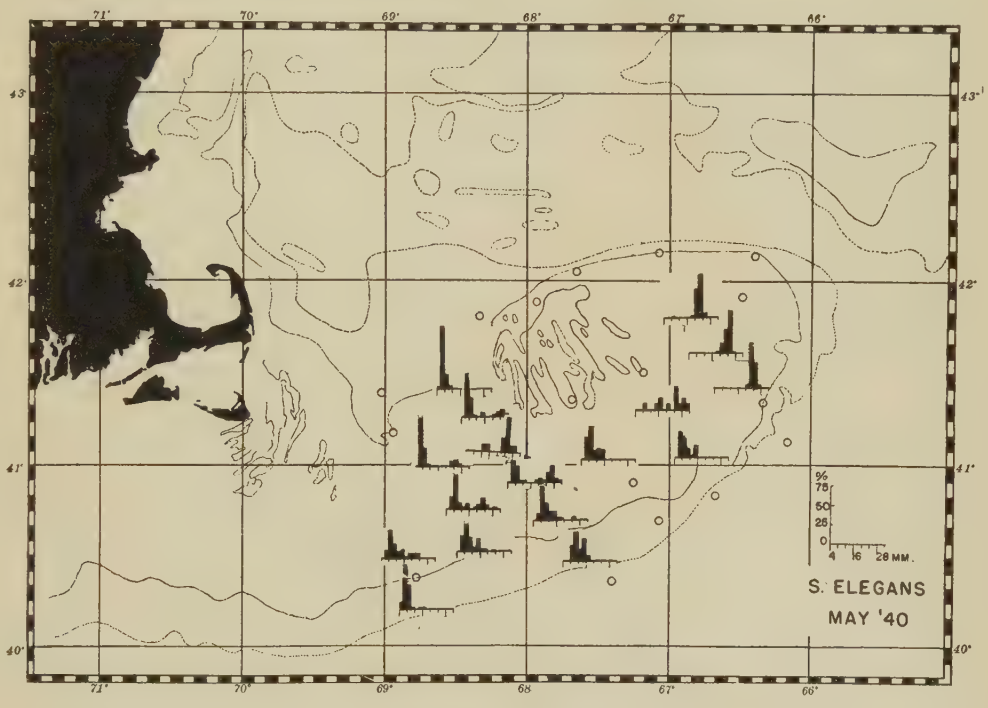

FIGURE 8. Length frequency distribution for individual stations in cruise of May 1940. Length measurements of *S. elegans* on percentage basis for individual stations, where numbers were sufficiently great.

From a study of the sequence of changes in the length and stage of maturity, as presented in Figure 9, information may be derived on the seasons of growth and reproduction of *Sagitta elegans* on Georges Bank. In the cruise of September 1939, the immature individuals were somewhat more numerous than either of the other stages, but both Stages II and III were well represented. The latter stages were much smaller in size than the corresponding groups taken during the spring months. The modal length of the mature Sagitta was only 16 mm. in September as compared to 23 mm. in the following March. In the January cruise Stages I and II were encountered in about equal numbers, but very few mature specimens were present. By March and April the majority of individuals had matured to the Stage III condition, and remnants of these animals were still found in diminishing numbers (and in smaller sizes) in the May and early

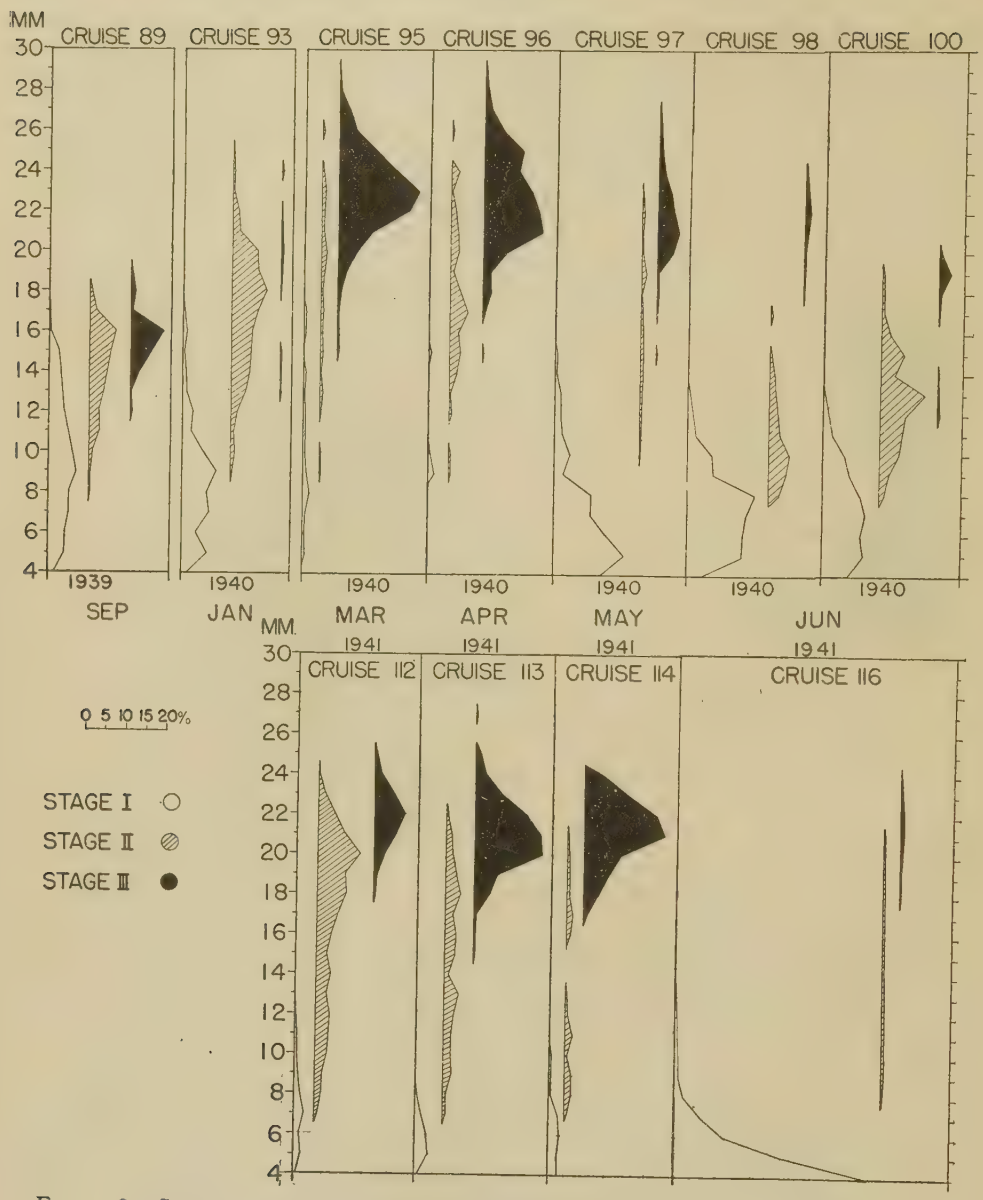

FIGURE 9. Stages of maturity and length frequency distribution for all cruises. Average values for *S. elegans* for all hauls in each cruise. Horizontal scale gives the percentage at each length, subdivided into the stages of maturity as indicated by the shading. Stage I—Immature, Stage II—Intermediate, Stage III—Mature.

June cruises. In May, however, the bulk of the catch consisted of immature specimens, and in the June cruises Stage I was also relatively the most abundant. Stage II was very scarce in May but appeared in increasing numbers (and in increasing sizes) in June. It is clear that this period of relative abundance of

immature animals corresponds to the time of great increase in the actual numbers of smaller sizes which was noted in the previous section.

The striking difference observed in the lengths of the mature Sagitta at various seasons of the year is correlated in a general way with temperature. The shortest modal length for Stage III (16 mm.) occurred in September when the temperature of the water in the Mixed Area surpassed 16° C., the highest for the year, and the greatest modal length (23 mm.) was observed in March when the temperature reached a minimum of about 2.5° C. (Table II). As temperature increased through June, the mature sagittae became shorter again. Russell (1932) reported similarly that an inverse relationship existed between the length of the mature *Sagitta elegans* and the temperature of the water in the Plymouth Area, and the same tendency was observed in the Irish Sea by Pierce (1941). The maximum temperature recorded by Russell was about the same as in the present investigation, but the average length of his Stage III animals was only about 10 mm. On the other hand, the minimum temperature off Plymouth did not fall below 8° C. and the average length of the adult Sagitta was about 12 mm. in February, about 16 mm. in April to May 1931, and about 20 mm. in May 1930. No animals of length greater than 22 mm. were taken by Russell, whereas specimens as long as 30 mm. were encountered on Georges Bank. Our observations therefore agree with Russell's in revealing an inverse relation between temperature and body length, but the actual values are quite different. We have no information at present as to the mechanisms which underlie these relationships.

The changes in the Sagitta population during the spring of 1941 as revealed by the four corresponding cruises of that year, agree in general with those of the previous spring. There is, however, consistent indication that growth and reproduction were delayed in 1941 (Figure 9, bottom), although no important difference in the temperature of the Mixed Area water occurred for the corresponding months (Table II). In March 1941, there were relatively many more Stage II animals and many fewer Stage III individuals than in March 1940. The mature Sagitta dominated the scene in April 1941 and persisted in much higher relative abundance in May of that year than had been the case in 1940. At the same time it is to be noted that no important increase in Stage I is indicated in May 1941, in marked contrast to the previous year. Nevertheless, by early June 1941 (Cruise 116) small-sized Stage I animals appeared in very great numbers both relatively and absolutely. Stage III was reduced to a small remnant at this time, and there was no sign of an increase in the numerical strength of Stage II as had occurred in the previous June. It may therefore be concluded that in 1941 both the appearance of mature adults and the production of the large spring crop of young took place about a month later than in 1940.

An attempt may be made from the foregoing information to ascertain the annual cycle of growth and reproduction of *Sagitta elegans* on Georges Bank, but it is obvious that the gaps in the record during months in which no data could be obtained prevent final conclusions from being reached. It seems almost certain, however, that the chief period of reproduction centers in April or May because of the very high proportion of mature adults in those months, and the appearance subsequently of very large numbers of small, immature individuals. The adults which produced these animals apparently die off in June and the new

crop of Sagitta matures during the summer to form a distinct generation of adults. This supposition is strongly supported by the fact that the Stage III animals found in September were of an entirely different size from those which had been prevalent in the spring. The graph (Fig. 9) suggests that the spring crop of young animals begins developing into Stage II in June with the possible appearance of a few of the new small-sized adults. The Stage I animals present in September may represent either the end of the spring and summer spawning or the beginning of the reproductive activity of the new generation of small adults. By January practically all of these small Stage III animals have disappeared and the immature individuals present are presumably their progeny. It seems safe to assume that the latter then slowly mature to produce the relatively large group of adults found in March and April, thus completing the cycle.

On the basis of the foregoing reasoning we may tentatively conclude that on Georges Bank *Sagitta elegans* undergoes one major period of reproduction during the spring months and that a distinct second generation is produced sometime in the late summer or autumn. It must be borne in mind, however, that during July and August and in the period between September and January, when no observations were made, another complete generation could have been formed. Russell (1932) believes that *Sagitta elegans* may complete a generation in as little as 43 days during the warmer months; and he has interpreted his data from the Plymouth Area as indicating that this species produces four or five generations during the course of the year. Pierce (1941), however, concludes that in the Irish Sea there is but one chief spawning period for *S. elegans* annually, extending from January through May. Our present data definitely indicate the existence of one major and one minor generation of this species in the Georges Bank area, but do not justify as yet any assumption that further generations occurred during the year.

Discussion

Information derived from the foregoing analyses of the distribution, growth, and reproduction of *Sagitta elegans* may now be examined as a contribution to the ecology of this species on Georges Bank particularly with reference to the currents of the region. Our previous knowledge of the occurrence of *S. elegans* off the New England coast has been summarized by Redfield and Beale (1940) in relation to their own studies of the sagittae in the Gulf of Maine. Although no special study of Georges Bank was undertaken by these authors, occasional stations on the Bank were occupied in the course of their survey, and at these stations large numbers of *S. elegans* were almost always encountered. These rich hauls contrasted sharply with the situation in the central area of the Gulf of Maine where the species was very scarce at practically every station. The explanation offered by Redfield and Beale is that water barren in respect to *Sagitta elegans* enters the Gulf each year and circulates through the central area so rapidly that sufficient time does not exist for large populations of this species to build up, even though the ecological conditions may be favorable in other respects. On Georges Bank, in contrast, these authors suggest that the water mass is sufficiently permanent to allow sagittae to accumulate and to further augment their number through effective reproduction.

The present investigation, based as it is on a much larger number of hauls on Georges Bank itself, not only tends to confirm the general suggestions of Redfield and Beale, but also provides strong evidence on the degree of permanence of the bank water. On the other hand, scrutiny of all the present data indicates that ecological factors other than simple transportation and accumulation play important roles in determining the distribution and abundance of the plankton. Redfield and Beale themselves point out that the central area of the Gulf of Maine supports a rich endemic population of Crustacea in spite of the fact that

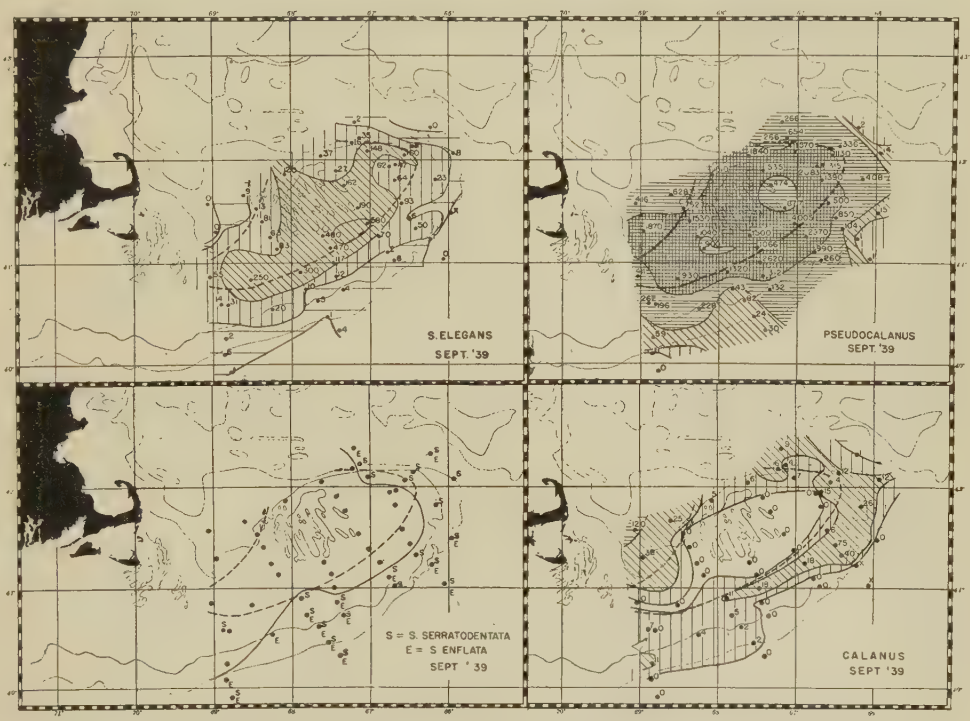

FIGURE 10. Comparison of the horizontal distribution of certain species of plankton in September 1939 (cruise 89). Distribution of *Sagitta elegans* is compared with that of *S. serratodentata*, *S. enflata*, *Pseudocalanus minutus*, and *Calanus finmarchicus*. Values shown are average numbers per cubic meter (per 10 cubic meters for *S. elegans*) for the whole water column for all stages of each species. Plankton Sampler hauls. Occurrence records for *S. serratodentata* and *S. elegans* are from the 75 cm. silk net hauls. The boundary of the Mixed Area is indicated by the heavy broken line. The symbol "X" represents a value of less than one individual per unit volume.

these animals are presumably subject to the same dislocating action of the currents as was invoked to explain the scarcity of *S. elegans* in that area.

The agreement of the distribution of *S. elegans* and of certain other species of plankton with the disposition of the water masses on Georges Bank during the present survey is well illustrated by the comparison for the cruise of September 1939, presented in Figure 10. Allusion has been made above to the close conformity of the main abundance of *Sagitta elegans* to the limits of the Mixed Area

for that period. Two other species of *Sagitta* appeared during the September cruise in sufficient abundance to permit significant analysis of distribution to be made, although numbers were so small that presence or absence alone has been indicated in the chart of their occurrence (Figure 10, lower left). It is seen that the distribution of *S. serratodentata* and *S. enflata* was confined to the southern and eastern margins of the Bank in distinct contrast to *S. elegans*. A remarkably close agreement is observed between the line limiting the occurrence of the former and the margin of the Mixed Area. *S. serratodentata* occurred farther up on the Bank than did *S. enflata* but neither had been carried into the Mixed Area at more than one or two points. This fact shows that a very small amount of water, if any, was entering the Mixed Area from the south or the east at the time of this cruise.

It is of value to compare this striking case of the separation of two morphologically similar species by the hydrographical condition on the Bank with an equally clear-cut reciprocity in the distribution of two copepods, *Pseudocalanus minutus* and *Calanus finmarchicus* (Figure 10, upper right and lower right). Pseudocalanus occurred at all stations on the Bank during this cruise in very large numbers except along the southern and northeastern margins, but concentrations of over 1000 individuals per cubic meter were limited to the central region and to the north central margin, this zone of abundance corresponding closely to the Mixed Area.

In the case of Calanus, on the other hand, no specimens whatsoever were taken in the central portion of the Bank and very low counts were obtained for this species at every station within the Mixed Area (Fig. 10). Similarly small catches of Calanus were made along the extreme southern margin of the Bank and to the northeast, but considerable numbers were taken at the stations to the north and west toward the Gulf of Maine, and in the zone between the Mixed Area and the southern margin of the Bank. The tongue of water, rich in Calanus, which appears extending across the eastern end of the Bank and curving south and then west, is a clear indication that at the time of this cruise a current carrying Calanus from the Gulf of Maine was flowing around the margin of the Mixed Area and forming a wedge between it and the water masses to the south. At the same time mixed water, barren in respect to Calanus, from the central eddy of the Mixed Area appears to have been draining off to the west down the middle of the Bank. *Calanus finmarchicus* therefore appears to be a species which can endure neither the homogeneous water of the Mixed Area nor the warm, saline conditions to the south, but which thrives in the water of the Gulf of Maine. Thus Calanus is similar to *S. serratodentata* and *S. enflata* in being sharply excluded from the Mixed Area of Georges Bank, whereas Pseudocalanus and *S. elegans* are chiefly abundant within it.

Although one could argue that even if *S. serratodentata* and *S. enflata* found their way on to Georges Bank, they could not survive there because of lower temperatures and salinities, it is impossible to invoke these factors as preventing the occurrence of *Calanus finmarchicus* in the central part of the Bank. As we have seen, the water of the Mixed Area is chiefly derived from the Gulf of Maine where Calanus is abundant, and the temperature and salinity values of this water are generally intermediate between those of the upper and lower strata of the Gulf. Some other factor must be found which could prevent the repro-

duction of Calanus in the Mixed Area or which could cause its destruction (or both), and which does not affect Pseudocalanus adversely. Perhaps sediment resulting from the turbulence in the Mixed Area is harmful to Calanus or possibly some essential element is lacking, such as accessible bottom water of low temperature (cf. Bigelow and Sears, 1939), or a necessary food organism. On the other hand, destruction of Calanus might be brought about chiefly by a predator, and as such *S. elegans* suggests itself for future study since it populates so abundantly the very area from which Calanus is removed.

Since other ecological influences besides the purely mechanical action of the current system are apparently controlling the occurrence of Calanus on Georges Bank, it seems probable that additional, as yet undetermined, factors may be important in accounting for the special richness of the population of *Sagitta elegans* in the Mixed Area. It now appears well established that the relative permanency of the bank waters makes possible the maintenance of an adequate breeding stock of *S. elegans* from one period of reproduction to another, but, in addition the data presented above indicate that the breeding and growth of this species is especially successful in the mixed bank water during the spring months, and perhaps at other seasons as well.

This suggestion that the great abundance of *Sagitta elegans* within the Mixed Area of Georges Bank is due not merely to mechanical accumulation but also to especially favorable local conditions, is supported by observations from other regions. Bigelow and Sears (1939) consider that this species is regularly endemic in the waters overlying the inner half of the continental shelf from Cape Cod south possibly to the offing of Chesapeake Bay. There are no large, permanent eddies in this area. Russell (1939) pointed out that *S. elegans* occurred in dominant numbers around the British Isles in areas where *mixed oceanic and coastal water* occurred. This author does not report any accumulation of the species in these areas through a mechanical action of the currents, but states that "The incursion of oceanic water gives rise to conditions in which a different plankton community, typified by *Sagitta elegans*, flourishes."

It is obviously of importance to inquire what characteristic of the mixed water of Georges Bank may make it an especially favorable environment for the reproduction and growth of *Sagitta elegans*. Temperature and salinity may apparently be ruled out since we have seen that the values for these factors are within the range of those found in the central region of the Gulf of Maine where this species is relatively scarce. Three other possibilities suggest themselves: (1) the turbulence and the attendant vertical homogeneity of the water, (2) the shallowness of the water, and (3) the existence of some element in the water originally derived from the shore or from the bottom. Although we do not have the means at present to decide between these, or other possibilities for Georges Bank, some evidence may be obtained from the ecological conditions of other regions of abundance for this species. Such comparison appears to eliminate vertical mixing of the water, *per se*, as particularly favorable since this condition does not characterize most of the other areas of occurrence of *S. elegans*. The waters of the continental shelf to the south, and of Massachusetts Bay to the north, are both definitely stratified during the warmer portion of the year. Furthermore, breeding was found to be unsuccessful in the Bay of Fundy (Huntsman and Reid, 1921) where vertical mixing is strong.

The shallowness of the water might be regarded as a beneficial condition for *S. elegans* since all observations agree that this species occurs chiefly in the shoal water of the continental shelf or of off-shore banks both along the North American coast and around the British Isles. It is doubted, however, whether the nearness of the bottom could be a *direct*, favorable influence (a) because there is no evidence that this species has any ecological dependence on the bottom, (b) because its distribution on Georges Bank showed no relation to the depth contours, and (c) because in other regions it has been found to be as abundant in water 100 m. to 200 m. deep as in shallower zones.

The third suggestion, that some beneficial derivative of the shore or the bottom occurs in the shallower water of the coastal areas or the banks, remains as a possible, though vague explanation. Fraser (1939) remarks with reference to Sagitta that plankton in general can withstand "fairly big" physical changes and hence the very distinct separation in the distribution of species means some biological change in the water masses. The very definite tendency for abundant populations of *S. elegans* to occur in shoal areas therefore suggests the presence in the water of some chemical element derived from the shore or bottom or some food organism dependent on the bottom, which does not exist in the water of deeper regions.

We conclude, therefore, that *Sagitta elegans* is chiefly abundant within the Mixed Area of Georges Bank, first, because the relative permanence of this water mass allows it to accumulate there; second, because water of harmfully high temperature and salinity from the south is excluded; and third, because some indirect influence from the shore or bottom, absent in the deeper water to the north, favors its reproduction and growth. It remains for the future to re-examine the water of the Mixed Area in order to ascertain what conditions of feeding or other circumstance, render this water particularly favorable for *S. elegans* and certain other types of plankton, and particularly unfavorable for other, closely related species.

Finally, it may be emphasized that the observations from the eleven cruises of this investigation have shown that Georges Bank supports a relatively abundant population of *S. elegans* throughout the year, and that the center of concentration of Sagitta was always found within the Mixed Area. Furthermore, the contours of the Sagitta population were shown to conform in general to the limits of the Mixed Area. Frequently this rich area was *completely surrounded* by water in which *S. elegans* was very scarce or absent. It appears then that this species is an adequate indicator for the presence of Mixed Area water. We know, as a result, that the eddy of homogeneous water on Georges Bank may be regarded as permanent to the extent to which the population of *Sagitta elegans* has been shown to maintain its integrity there from season to season. It is of special interest to note that a nucleus of this species apparently retained its position on the Bank throughout the winter, during the period when the breakdown of stratification in the surrounding areas might be expected to make possible a flow of Gulf water directly across the Bank. The bubble of mixed water on the Bank therefore either fails to be dislodged by hydrographic forces, or is renewed so slowly that the population of Sagitta is able to maintain itself within the Mixed Area despite the water movement. By similar application of

these findings, it should be feasible to employ *S. elegans* as an indicator to trace the movements of Mixed Area water in future studies of the ecology of the region.

Summary

1. The quantitative distribution, size, and stages of maturity of *Sagitta elegans* in the waters of Georges Bank have been determined from plankton hauls made on a network of stations occupied during 11 cruises from September 1939, to June 1941.

2. The area of relatively homogeneous water overlying the central portion of Georges Bank was found to change in extent from cruise to cruise, but to be sharply delineated from the surrounding stratified water masses, and has been designated as the "Mixed Area."

3. The abundance of *S. elegans* varied in individual hauls from a maximum of 165 specimens per cubic meter to zero, but averaged more than 10/m.^{3} at stations within the Mixed Area. The deeper hauls were numerically richer than the shallow hauls at more than half the stations. The existence of a diurnal vertical migration was revealed in certain cases, but varied greatly from cruise to cruise.

4. In horizontal distribution the greatest concentrations of *S. elegans* were found within the central homogeneous water mass of the Bank and a close agreement was disclosed between the contours of abundance and the boundary line of the Mixed Area. A center of abundance for this species existed on the Bank throughout the year.

5. The modal length of the mature sagittae increased from 16 mm. in September, when the water temperature was the highest, to 23 mm. in March, when the temperature was at the minimum for the year. Some specimens as long as 30 mm. were encountered.

6. The chief period of reproduction for *S. elegans* on Georges Bank centered in April in 1940 and in May in 1941. Following these periods very large numbers of small, immature individuals appeared. Evidence is presented that a distinct, second generation was produced during the late summer or autumn.

7. *S. serratodentata* and *S. enflata*, which were taken chiefly in the September cruise, occurred entirely outside the margin of the Mixed Area. Comparison is made with a similar case of reciprocal distribution found for two species of copepods: *Calanus finmarchicus*, which was excluded from the Mixed Area, and *Pseudocalanus minutus*, which was chiefly abundant within the Area.

8. Our observations support the suggestion of Redfield and Beale that *S. elegans* tends to accumulate on Georges Bank because of the relative absence of dislocating currents. However, reasons are advanced for believing that other characteristics of the Mixed Area water should be scrutinized as being particularly favorable for the growth and reproduction of *S. elegans*, and particularly unfavorable for the existence of other species of plankton.

9. The persistence of the population of *S. elegans* on Georges Bank throughout the year is a valuable index of the degree of permanence of the homogeneous bank water. The species recommends itself as an indicator for tracing movements of the Mixed Area water in future studies.

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Report of the year 1942

THE WOODS HOLE OCEANOGRAPHIC INSTITUTION

REPORT FOR THE YEAR
1942

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Thomas Barbour, Museum of Comparative Zoölogy, Cambridge, Mass.
Jno. A. Fleming, Carnegie Institution, Washington, D. C.
Frank B. Jewett, 195 Broadway, New York, N. Y.
Albert E. Parr, American Museum of Natural History, New York, N. Y.
Elihu Root, Jr., 31 Nassau Street, New York, N. Y.

To serve until 1945

The Commandant (Admiral R. R. Waesche), U. S. Coast Guard, for the time being, Washington, D. C.
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Henry L. Shattuck, 50 Federal Street, Boston, Mass.
T. Wayland Vaughan, 3333 P Street, Washington, D. C.

To serve until 1943

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Columbus O'D. Iselin, Woods Hole Oceanographic Institution, Woods Hole, Mass.
Lawrason Riggs, 120 Broadway, New York N. Y.

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Lawrason Riggs, Treasurer, 120 Broadway, New York, N. Y.
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Thomas Barbour, Museum of Comparative Zoölogy, Cambridge, Mass.
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Isaiah Bowman, Johns Hopkins University, Baltimore, Md.
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Henry L. Shattuck, 50 Federal Street, Boston, Mass.
T. Wayland Vaughan, 3333 P Street, Washington, D. C.

III. REPORT OF THE TREASURER

To the Members and Trustees of the
Woods Hole Oceanographic Institution:

Gentlemen:

The accounts for the year 1942 have been audited by Messrs. Stagg, Mather & Hough, Public Accountants.

During the year bonds costing $89,395.50 were redeemed at a net profit of $2,241.54 (after adding the applicable reserve for amortization).

There was invested during the year, in bonds $80,412.50 and in stocks $15,017.24.

In addition to the amount of $24,656.01 due from the General Account to the Special Income Account at the end of 1941, there was withheld during the year further special income of $2,512.50 making a total of $27,168.51 due this fund at the end of 1942. The continued holding of these funds due special income in the general fund was necessitated by the unavoidable delay in the payment of our vouchers on Government projects.

The indebtedness of the Government to the Institution increased from $42,471.55 at the end of 1941 to $69,335.10 at the end of 1942.

There was billed to the Government during the year $202,718.89 for disbursements incurred for the Government and $40,562.96 allowance for general overhead. In addition to this a large part of our income is being used for the benefit of the Government without reimbursement other than that mentioned above.

The entire appropriation of $4,800.00 for the reserve for periodic replacements was added to that fund. This fund has grown very satisfactorily and amounted to $47,592.94 in market value at the end of the year.

The total expenses of the Institution (not including disbursements for the Government and after applying the allowance from the Government for overhead) was under income, including special income, by $29,117.12.

The cash position was not improved due to the increase in accounts receivable from the Government.

The gross income on the securities in the Endowment Fund excluding special income (extra dividends) was about .0445 of cost and .0497 of value at the end of the year.

Summaries of the balance sheet and comparative income and expense statement for 1941 and 1942 are appended. The expenditures for the Government are not included.

Respectfully submitted,

Lawrason Riggs
Treasurer

BALANCE SHEET

ENDOWMENT FUND ASSETS

	Quoted Market Value		
Endowment fund assets:			
Bonds (less reserve for amortization of bond premiums, $7,717.21)	$1,134,247.25	$1,206,862.79	
Stocks	$1,082,396.88	1,261,306.48	
Cash		5,703.25	$2,473,872.52
Reserve fund assets:			
Bonds	$ 4,517.50	$ 4,697.50	
Stocks	$ 11,846.13	19,099.02	
Cash		6,988.03	30,784.55
			$2,504,657.07

PLANT ASSETS

Laboratory plant:		
Land	$ 27,072.32	
Buildings	323,213.12	
Laboratory equipment	21,361.93	
Crew room equipment	557.95	
Library	13,500.00	$ 385,705.32
Ship "Atlantis":		
Construction	$ 218,674.47	
Equipment	27,298.50	245,972.97
Ship "Anton Dohrn"		17,145.67
Small Boat and equipment		6,570.55
		$ 655,394.51
Depreciation fund assets (for periodic replacements):		
Bonds (quoted market value $31,882.50)	$ 32,035.00	
Cash	15,710.44	47,745.44
		$ 703,139.95

CURRENT ASSETS

Current funds—Cash	$ 4,350.07	
Less—Due to special income fund (below)	27,168.51	$ *22,818.44*
Special income assets:		
Investments:		
Bonds (quoted market value $7,900.00)	$ 7,900.00	
Stocks (quoted market value $2,621.88)	2,809.46	
Cash (including $27,168.51 due from current funds—Cash) (above)	27,324.53	38,033.99
Accounts receivable:		
Navy Project No. 1	$ 4,515.28	
Navy Projects Nos. 2 to 8	64,819.82	
Mess Accounts	407.60	69,742.70
		$ 84,958.25
Total assets		$3,292,755.27

DECEMBER 31, 1942

ENDOWMENT FUNDS

Endowment fund—General		$2,000,000.00	
Endowment fund—For upkeep of plant		419,419.96	
		$ 2,419,419.96	
Add—Accumulated net gain on securities called or sold		54,452.56	$2,473,872.52
Reserve fund		$ 23,395.57	
Add—Accumulated reserve fund income		7,388.98	30,784.55
			$2,504,657.07

PLANT FUND

Plant fund general		$ 607,642.13	
Plant fund reserve—Transferred in prior years from current surplus	$ 46,952.38		
Add—Appropriation from current funds: Library	800.00	47,752.38	$ 655,394.51
Reserve for periodic replacements			47,745.44
			$ 703,139.95

CURRENT LIABILITIES AND SURPLUS

Accounts payable:			
Institution		$ 5,135.34	
Navy Projects		6,382.41	$ 11,517.75
Capital surplus:			
Balance at December 31, 1941		$ 44,323.38	
Add—Excess of income over expenses for the year ended December 31, 1942		29,117.12	73,440.50
			$ 84,958.25
Total funds, current liabilities and surplus			$3,292,755.27

STATEMENT OF INCOME FROM ENDOWMENT FUND INVESTMENTS AND FROM CURRENT ASSETS, AND EXPENSES (LESS ALLOWANCE FOR GENERAL OVERHEAD BY THE U. S. GOVERNMENT) APPLICABLE THERETO, INCLUDING EXPENDITURES FOR PERMANENT PLANT ADDITIONS

FOR THE YEARS ENDED DECEMBER 31, 1942 AND DECEMBER 31, 1941

		Year Ended December 31, 1942			*Year Ended December 31, 1941*		
Income:							
From endowment funds:							
Interest (including in 1942 special income of $200.00)		$51,777.88			$ 54,384.34		
Dividends (including in 1942 special income of $3,268.07)		60,849.84			64,260.93		
Net profit on bonds called or sold during the year, special income		—			390.40		
			$112,627.72			$119,035.67	
Less—Custodian fees and expenses			1,589.87			1,559.16	
Total income				$111,037.85			$117,476.51
Expenses:	*Budget—1942*						
Upkeep of plant:							
Upkeep of buildings and grounds	$ 7,847.00	$17,365.22			$ 8,801.62		
Upkeep of "Atlantis"	10,000.00	9,393.41			9,298.14		
Upkeep of "Asterias"	200.00	611.34			550.53		
Upkeep of "Anton Dohrn"	—	3,369.28			4,225.93		
Insurance	6,062.00	7,129.83			7,311.29		
Depreciation	4,800.00	4,800.00			4,800.00		
	$ 28,909.00		$ 42,669.08			$ 34,987.51	
Operating expenses:							
Operation of "Atlantis"	$ 34,664.00	$26,263.45			$ 28,675.41		
Operation of "Asterias"	825.00	891.75			1,000.33		
Operation of "Anton Dohrn"	—	4,547.94			3,347.75		
Scientists' salaries	27,245.00	23,218.70			23,720.56		
Scientific supplies and equipment	4,500.00	3,584.28			7,145.91		
Traveling expenses	475.00	881.28			925.28		
Publications	3,000.00	1,846.37			1,667.59		
Administration	7,600.00	14,171.23			9,048.31		
Amortization of bond premiums	2,500.00	1,288.63			1,739.13		
Contribution to retirement fund	1,500.00	1,382.83			1,543.22		
Interest on bank loan	500.00	152.22			35.16		
Staff meetings	50.00	—			33.13		
Mess (net)	—	785.93			—		
	$ 82,859.00		79,014.61			78,881.78	
Total expenses	$111,768.00		$121,683.69			$113,869.29	
Expenditures for permanent plant additions:							
Additions to library	$ 800.00	$ 800.00			$ 800.00		
Reconditioning the "Anton Dohrn"	—	—			17,145.67		
	$ 800.00		800.00			17,945.67	
(Not budgeted)	$ 682.00		—			—	
	$113,250.00		$122,483.69			$131,814.96	
Less—Allowance for general overhead, by the U. S. Government			40,562.96			21,277.64	
				81,920.73			110,537.32
Excess of income over expenses				$29,117.12			$6,939.19

IV. THIRTEENTH ANNUAL REPORT OF THE DIRECTOR FOR THE YEAR 1942

BY C. O'D. ISELIN

Because our facilities during the past year have become more and more used for a variety of research programs in which oceanography has a practical application in warfare, this report can only summarize a small part of the work which has been in progress. The laboratory has been an exceedingly busy place, both in winter and in summer. Many investigators have been working here who do not ordinarily concern themselves with oceanography. It is also good to report that much of the new work is increasing our knowledge of the sea and will be of lasting importance to oceanography at the end of the war.

Research Vessel Atlantis

It was decided early in the year to send the "Atlantis" to the Caribbean or the Gulf of Mexico in order to secure observations which would be used for studying various phases of the heat and water vapor exchange between the sea and the atmosphere. The preparations for this cruise consumed all of January, for there was much to be done to outfit the vessel for work at sea under war time conditions.

While cruising down the coast one night early in February the "Atlantis" was struck a glancing blow by a tanker. The collision occurred near Cape Hatteras and the "Atlantis" was able to put in to Norfolk under her own power. The starboard rail was badly bent inward for about 40 feet while a number of frames in the middle part of the vessel were sprung and a fair size hole was made above the waterline just aft of the main rigging.

Fortunately we were able to persuade the Newport News Shipbuilding and Drydock Company to undertake the repairs, and within two weeks the "Atlantis" was as good as new and able to proceed down the coast. The cost of the repairs, amounting to about $10,000, was entirely taken care of by insurance.

By the time the "Atlantis" reached Key West the Caribbean had become unsafe, so it was decided to work in the central part of the Gulf of Mexico. During March and April, under Mr. Woodcock's direction, a very complete study of diurnal temperature variations near the surface was carried out there. It is believed that these data are unique. They have enabled us to study the relative importance of the several variables which combine to produce vertical temperature changes at shallow levels. Further observations on the effect of the wind on the pattern of convectional circulation near the surface were also secured.

Early in May the "Atlantis" put in at Galveston for fuel and stores. While she was in port enemy submarines began to operate in the Gulf of Mexico. Insurance rates went sky high and it was decided that further work at sea would be impractical. With the approach of the hurricane season it became possible to move her to Lake Charles, Louisiana, where she could be moored in a much better protected spot. She has been there ever since.

Captain F. S. McMurray of the "Atlantis" left to take command of a cargo steamer and others left for various reasons so that by the end of the year the crew was reduced to only three men in place of the usual seventeen. Meanwhile, the vessel has been put in excellent condition, the entire deck having been caulked and all interior surfaces cleaned off and repainted. Thus upkeep is now reduced to a minimum.

Research Vessel Anton Dohrn

Throughout the year the "Anton Dohrn" has been used along the coast, between Maine and North Carolina. She has worked mainly by day, returning to port nearly every night. Such operations involve no more risk than that accepted by the fishing fleet and it has seldom seemed necessary to take out special insurance. Thus through the "Anton Dohrn" we have continued to secure some observations, although at relatively short distances from the land. At the same time it has been possible to make many minor improvements so that she has been gradually transformed into a most useful and convenient vessel.

Motor Boat Asterias

The "Asterias" has likewise been employed continuously in local waters. She has been used for a wide variety of purposes, and there has been much building in and ripping out of equipment. By the end of the war she will bare many honorable scars.

Laboratory Equipment

Besides the expansion in our shop facilities, begun last year and continued during 1942, we have made considerable additions to the furniture and fixtures in the main building. A big chemical table was built into one of the second floor laboratories, so that we now have three large rooms equipped for chemical work. In several cases, by means of temporary partitions, extra laboratory space has been gained. The storage facilities in the attic have been improved and increased. At the same time of course it has been necessary to increase our stock of commonly used laboratory equipment. Thus, out of our own funds, we have in many ways improved the usefulness

of the laboratory, both during the present emergency and for the years to come.

Personnel

At the Annual Meeting of the Trustees Dr. Alfred C. Redfield was appointed Associate Director and he arranged for a year's leave of absence from Harvard University. At the same time two new staff appointments were made: Dr. E. Bright Wilson, Jr. as Associate in Chemistry and Mr. Alfred H. Woodcock as Associate in Oceanography.

Fellowship holders who worked at the laboratory during the summer were: Dr. Robert Pyle, Dr. George Whiteley, Mr. William Schallek, Mr. George Scott and Mr. Donald B. Johnstone.

Visiting investigators included Dr. Lawrence Irving, Dr. Pierre Scholander, Dr. George Edwards, Dr. and Mrs. Earl Myers, Mr. Gerome Brush, Mr. D. W. Bishop and Mr. D. W. Claff.

During the year a number of persons connected with the laboratory have gone on active duty with the armed forces: Lieut. Commander Floyd Soule in the Coast Guard Reserve, Lieut. Francis Kinnicutt in the Naval Reserve, Lieut. T. N. Kelley in the Coast Guard Reserve, Lieut. (j.g.) Mary Sears in the Women's Reserve, U. S. Naval Reserve, Ensign Herbert Schroeder in the Coast Guard Reserve, Second Lieut. George Kelly in the Marine Corps Reserve and Private William Condon in the Army.

Library

Our contribution to the library of the Marine Biological Laboratory was again $800. This sum was spent as follows: 18 subscriptions to current journals and monographs, 15 books purchased, 2 back sets completed by purchase, 3 back sets partly completed by purchase and 95 volumes bound. Seventeen current journals were received in exchange for our publications and 9 were received as gifts.

Publications

Number 2 of Volume IX of "Papers in Physical Oceanography and Meteorology" appeared.

The following contributions were published during 1942:

No. 184. J. L. Hough. Sediments of Cape Cod Bay, Massachusetts. *Jour. Sedimentary Petrology*, Vol. 12, No. 1, pp. 10–30.

No. 286. Floyd M. Soule. Physical Oceanography—The Grand Banks Region and the Labrador Sea in 1940. Excerpt from International Ice Observation and Patrol Service in the North Atlantic Ocean. Season of 1940, *U. S. Coast Guard Bull.*, pp. 36–89.

No. 291. H. R. Seiwell. An Analysis of Vertical Oscillations in the Southern North Atlantic. *Proc. Am. Philos. Soc.*, Vol. 85, No. 2, pp. 136–158.

No. 292. Theodor Von Brand and Morris W. Rakestraw. Decomposition and Regeneration of Nitrogenous Organic Matter in Sea Water. IV, Interrelationship of Various Stages; Influence of Concentration and Nature of Particular Matter. *Biol. Bull.*, Vol. LXXXI, No. 1, pp. 63–69.

No. 297. Fred B. Phleger, Jr. Foraminifera of Submarine Cores from the Continental Slope, Part 2. *Bull. Geol. Soc. Amer.*, Vol. 53, pp. 1073–1098.

No. 304. Richard E. Lee. The Occurrence of Female Swordfish in Southern New England Waters, with a Description of their Reproductive Condition. *Copeia*, No. 2, pp. 117–119.

No. 305. R. B. Montgomery and M. J. Pollak. Sigma-T Surfaces in the Atlantic Ocean. *Sears Found. Jour. Mar. Res.*, Vol. V, No. 1, pp. 20–27.

No. 307. Robert W. Pennak. Harpacticoid Copepods from some Intertidal Beaches near Woods Hole, Massachusetts. *Trans. Am. Micro. Soc.*, Vol. LXI, No. 3, pp. 274–285.

No. 308. Alfred C. Redfield. The Processes Determining the Concentration of Oxygen, Phosphate and other Organic Derivatives within the Depths of the Atlantic Ocean. *Papers in Physical Oceanography and Meteorology*, Vol. IX, No. 2, pp. 1–22.

No. 309. William C. Schroeder. Results of Haddock Tagging in the Gulf of Maine from 1923 to 1932. *Sears Found Jour. Mar. Res.*, Vol. V, No. 1, pp. 1–19.

No. 310. W. H. Johnson. Effect of Light on Copepods as Food for Passamaquoddy Herring. *J. Fish. Res. Bd. Can.* 5(4), pp. 365–376.

No. 311. Charles E. Renn. Demonstration of Labyrinthula Parasite in Eel-Grass from the Coast of California. *Science*, Vol. 95, No. 2457, pp. 122.

No. 312. C. O'D Iselin. Interaction Between the Hydrosphere and the Atmosphere. *Trans. N. Y. Acad. Sci.*, Series II. Vol. 4, No. 3, pp. 99–106.

No. 314. Earl H. Myers. Rate at which Foraminifera are Contributed to Marine Sediments. *Jour. Sedimentary Petrology*, Vol. 12, No. 2, pp. 92–95.

No. 315. Gordon A. Riley. The Relationship of Vertical Turbulence and Spring Diatom Flowerings. *Sears Found. Jour. Mar. Res.*, Vol. V, No. 1, pp. 67–87.

No. 316. Earl H. Myers. Biological Evidence as to the Rate at which Tests of Foraminifera are Contributed to Marine Sediments. *Journal of Paleontology*, Vol. 16, No. 3, pp. 397–398.

No. 317. Richard E. Lee. The Hydrophysics of the Broad-billed Swordfish, *Xiphias gladius L. Biol. Bull.*, Vol. LXXXII, No. 3, pp. 401–412.

No. 318. Athelstan F. Spilhaus. Maps of the Whole World Ocean. *Geog. Rev.*, Vol. XXXII, No. 3, pp. 431–435.

No. 320. Olga Hartman. Report on the Scientific Results of the Atlantis Expeditions to the West Indies under the Joint Auspices of the University of Havana and Harvard University. The Polychaetous Annelida. *Mem. Soc. Cub. Hist. Nat.*, Vol. 16, No. 2, pp. 89–104.

No. 321. Theodor Von Brand, Norris W. Rakestraw and J. William Zabor. Decomposition and Regeneration of Nitrogenous Organic Matter in Sea Water. V. Factors Influencing the Length of the Cycle; Observations upon the Gaseous and Dissolved Organic Nitrogen. *Biol. Bull.*, Vol. 83, No. 2, pp. 273–282.

No. 322. Fenner A. Chace, Jr. Reports on the Scientific Results of the Atlantis Expeditions to the West Indies, under the Joint Auspices of the University of Havana and Harvard University. The Anomuran Crustaces. I Galatheida (Families Chirostylidae, Galatheidae and Porcellanidae). *Torreia*, No. 11, pp. 1–106.

No. 324. Richard E. Lee. Notes on the Color Changes of the Sea Robin (*Prionotus strigatus* Cuvier) with Special Reference to the Erythrophores. *Jour. Exper. Zool.*, Vol. 91, No. 2, pp. 131–153.

Scientific Program

The study of the "Atlantis" plankton collections from Georges Bank continued throughout the year under Dr. Clarke's direction. Mr. Bumpus devoted his time to this work until the spring when Miss Delabarre took over. Dr. Sears also devoted much of her time to plankton studies. Although no new collections were secured, except those brought back from Peru in April by Dr. Sears, the existing material was more than sufficient to keep the available staff busy. During the summer months Dr. Whiteley cooperated in the identification and enumeration of the larger forms. Considerable progress was made in the preparation of final reports on various phases of all of this work.

Dr. Waksman and Dr. Carey resumed their studies of marine bacteria during the summer, assisted by Mr. Johnstone. They studied the role of bacteria in the transformation of copper in the sea and the effect of copper on bacterial development. These investigations have been continued by Mr. Johnstone at Rutgers.

Dr. Irving and his many assistants and associates have continued working on the rate of metabolism of marine organisms. Thus all summer we had an active physiological group in the laboratory.

Dr. Pyle, a fellowship holder, worked on the development of the sinus gland in crustacea. Mr. Lee, another fellowship holder, spent the summer completing the work he had begun the previous year. He managed to complete two reports before taking a position at New York University. Mr. Scott working under Dr. Redfield's direction studied the relationship of the mineral environment on the chemical composition of living cells. He found that the amounts of calcium, sodium, magnesium and potassium in a mineral environment determine the ratio and concentration of these ions combined by the protoplasm in an algal cell. Finally, Dr. and Mrs. Myers, as visiting investigators, were busy with their large collection of foraminifera from the Dutch East Indies. In September they left Woods Hole to work at the Hopkins Marine Station in California.

This very brief summary of the biological work in progress here during the past year should serve to indicate that in spite of the war we have not

forgotten our obligation to support and to encourage the study of the ocean in all its aspects. Although the physical side of our program has been much less active, this still remains a laboratory where all qualified investigators who are interested in the sea are welcome. It is believed that it will not become necessary to change this policy and that the only serious restriction to pure oceanography is the limitation which the war has imposed on field observations.

V. STAFF

(As of December 31, 1942)

COLUMBUS O'D. ISELIN, Associate Professor of Physical Oceanography, Harvard University, and Assistant Curator of Oceanography Museum of Comparative Zoölogy; Director.

ALFRED C. REDFIELD, Professor of Physiology, Harvard University; Associate Director.

CORNELIA L. CAREY, Assistant Professor of Botany, Barnard College; Associate in Marine Bacteriology.

GEORGE L. CLARKE, Associate Professor in Biology, Harvard University; Marine Biologist.

WILLIAM MAURICE EWING, Associate Professor in Geophysics, Lehigh University; Associate in Submarine Geology.

BOSTWICK H. KETCHUM, Associate in Marine Biology.

RAYMOND B. MONTGOMERY, Lecturer on Maritime Meteorology and Oceanography, Assistant Professor of Meteorology, New York University; Physical Oceanographer.

ALBERT E. PARR, Director of American Museum of Natural History, New York; Associate in Oceanography.

FRED B. PHLEGER, JR., Associate Professor of Geology, Amherst College; Submarine Geologist.

NORRIS W. RAKESTRAW, Associate Professor of Chemistry, Brown University; Chemical Oceanographer.

CHARLES E. RENN, Tutor in Biology and Instructor in Sanitary Engineering, Harvard University; Associate in Marine Bacteriology.

GORDON A. RILEY, Marine Biologist, Yale University; Marine Physiologist.

C.-G. ROSSBY, Professor of Meteorology, University of Chicago, and Assistant Chief, U.S. Weather Bureau, Washington, D. C.; Associate in Physical Oceanography.

MARY SEARS, Instructor in Zoölogy, Wellesley College; Planktonologist.

H. R. SEIWELL, Physical Oceanographer.

FLOYD M. SOULE, Principal Physical Oceanographer, U. S. Coast Guard; Associate in Physical Oceanography.

ATHELSTAN F. SPILHAUS, Associate Professor of Meteorology, Chairman of the Department of Meteorology, New York University; Physical Oceanographer.

HENRY C. STETSON, Research Associate in Paleontology, Museum of Comparative Zoölogy; Submarine Geologist.

SELMAN A. WAKSMAN, Microbiologist, New Jersey Agricultural Experiment Station; Marine Bacteriologixt.

EDMOND E. WATSON, Assistant Professor of Physics, Queen's University; Physical Oceanographer.

E. BRIGHT WILSON, JR., Associate Professor of Chemistry, Harvard University; Associate in Chemistry.

ALFRED H. WOODCOCK, Assiociate in Oceanography.

CHARLOTTE S. GRIFFIN, Secretary to the Associate Director.

HELEN F. PHILLIPS, Secretary to the Director.

WILLIAM C. SCHROEDER, Business Manager.

ETHELYN T. BIRD, Secretary.

WILLIAM SCHROEDER, Superintendent of Buildings and Grounds.

List of Contributions

LIST OF CONTRIBUTIONS

FROM THE WOODS HOLE OCEANOGRAPHIC INSTITUTION

For a list of Contribution Numbers 1 to 100 see previous issues from 1933 to 1940. A separate list of these numbers may be obtained on request.

No. 101. George L. Clarke. Light Penetration in the Western North Atlantic and its Application to Biological Problems. *Rapp. et Proc. Verb. Cons. Perm. Internat. Exp. Mer.*, Vol. 101, Pt. 2, pp. 1–14. 1936.

No. 102. C.-G. Rossby and R. B. Montgomery. I. On the Frictional Force between Air and Water and on the Occurrence of a Laminar Boundary Layer next to the Surface of the Sea. C.-G. Rossby. II. Measurements of Vertical Gradient of Wind over Water. R. B. Montgomery. III. Transport of Surface Water due to the Wind System over the North Atlantic. R. B. Montgomery. *Papers In Physical Oceanography and Meteorology*, Vol. IV, No. 3, pp. 1–30. 1936.

No. 103. H. C. Stetson. Dredge-Samples from the Submarine Canyons between the Hudson Gorge and Chesapeake Bay. *Trans. Am. Geophys. Union*, Seventeenth Annual Meeting, pp. 223–225. 1936.

No. 104. Columbus Iselin. The Influence of the Mediterranean Outflow at Mid-Depths in the Sargasso Sea. *Trans. Am. Geophys. Union*, Seventeenth Annual Meeting, pp. 219–221. 1936.

No. 105. R. B. Montgomery. Computation of the Transport of Surface-Water due to the Wind-System over the North Atlantic. *Trans. Am. Geophys. Union*, Seventeenth Annual Meeting, pp. 225–229. 1936.

No. 106. Margaret Hotchkiss and Selman A. Waksman. Correlative Studies of Microscopic and Plate Methods for Evaluating the Bacterial Population of the Sea. *Jour. Bacteriology*, Vol. 32, No. 4, pp. 423–432. 1936.

No. 107. Edward H. Smith, Floyd M. Soule, Olav Mosby. The "Marion" and "General Greene" Expeditions to Davis Strait and Labrador Sea under Direction of the United States Coast Guard 1928-1931-1933-1934-1935. Scientific Results. Part 2. Physical Oceanography. *U. S. Coast Guard Bull.*, No. 19, pp. i-vi, pp. 1–259. 1937.

No. 108. Columbus Iselin. Study of the Circulation of the North Atlantic Ocean. *Papers in Physical Oceanography and Meteorology*, Vol. IV, No. 4, pp. 1-101. 1936.

No. 109. Charles J. Fish. The Biology of *Oithona similis* in the Gulf of Maine and Bay of Fundy. *Biol. Bull.*, Vol. LXXI, No. 1, pp. 168–187. 1936.

No. 110. Norris W. Rakestraw. The Occurrence and Significance of Nitrite in the Sea. *Biol. Bull.*, Vol. LXXI, No. 1, pp. 133–167. 1936.

No. 111. Charles E. Renn. The Eel-Grass Situation along the Middle Atlantic Coast. *Ecology*, Vol. XVIII, No. 2, pp. 323-325. 1937.

No. 112. George L. Clarke and Donald J. Zinn. Seasonal Production of Zoöplankton off Woods Hole with Special Reference to *Calanus finmarchicus*. *Biol. Bull.*, Vol. LXXIII, No. 3, pp. 464–487. 1937.

No. 113. G. H. Parker. The Reactivation by Cutting of Severed Melanophore Nerves in the Dogfish Mustelus. *Biol. Bull.*, Vol. LXXI, No. 2, pp. 255–258. 1936.

No. 114. Philip H. Mitchell, Kurt Buch and Norris W. Rakestraw. The Effect of Salinity and Temperature upon the Dissociation of Cresol Red and Phenol Red in Sea Water. *Jour. d. Conseil*, Vol. XI, No. 2, pp. 183–189. 1936.

No. 115. C.-G. Rossby. Dynamics of Steady Ocean Currents in the Light of Experimental Fluid Mechanics. *Papers in Physical Oceanography and Meteorology*, Vol. V, No. 1, pp. 1–43. 1936.

No. 116. Charles P. Winsor and Lionel A. Walford. Sampling Variations in the Use of Plankton Nets. *Jour. d. Conseil*, Vol. XI, No. 2, pp. 190–204. 1936.

No. 117. Charles E. Renn. Persistence of the Eel-Grass Disease and Parasite on the American Atlantic Coast. *Nature*, Vol. 138, p. 507. 1936.

No. 118. H. R. Seiwell. Relationship of Minimum Oxygen Concentration to Density of the Water Column in the Western North Atlantic. *Gerlands Beiträge zur Geophysik*, Vol. 50, pp. 302–306. 1937.

No. 119. Henry B. Bigelow and William C. Schroeder. Supplemental Notes on Fishes of the Gulf of Maine. *Bull. Bur. Fisheries*, Vol. XLVIII, Bull. No. 20, pp. 319–343. 1936.

No. 120. Norris W. Rakestraw and Alexander Hollaender. Photochemical Oxidation of Ammonia in Sea Water. *Science*, Vol. 84, No. 2185, pp. 442–443. 1936.

No. 121. Phil E. Church. Temperatures of the Western North Atlantic from Thermograph Records. *Publ. Scientifique*, No. 4, pp. 1–32. 1937.

No. 122. Alfred C. Redfield. An Ecological Aspect of the Gulf Stream. *Nature*, Vol. 138, p. 1013. 1936.

No. 123. Theodor von Brand. Observations upon the Nitrogen of the Particulate Matter in the Sea. *Biol. Bull.*, Vol. LXXII, No. 1, pp. 1–6. 1937.

No. 124. Selman A. Waksman and Margaret Hotchkiss. Viability of Bacteria in Sea Water. *Jour. Bacteriology*, Vol. 33, No. 4, pp. 389–400. 1937.

No. 125. Albert Eide Parr. A Contribution to the Hydrography of the Caribbean and Cayman Seas. Based upon the observations made by the Research Ship "*Atlantis*", 1933–34. *Bull. Bingham Oceanographic Coll.*, Vol. V, Art. 4, pp. 1–110. 1937.

No. 126. J. H. Welsh and F. A. Chace, Jr. Eyes of Deep Sea Crustaceans. I. Acanthephyridae. *Biol. Bull.*, Vol. LXXII, No. 1, pp. 57–74. 1937.

No. 127. Alfred C. Redfield, Homer P. Smith and Bostwick Ketchum. The Cycle of Organic Phosphorus in the Gulf of Maine. *Biol. Bull.*, Vol. LXXIII, No. 3, pp. 421–443. 1937.

No. 128. William F. Wrath. Contamination and Compaction on Core Sampling. *Science*, Vol. 84, No. 1289, pp. 537–538. 1936.

No. 129. Theodor von Brand, Norris W. Rakestraw and Charles E. Renn. The Experimental Decomposition and Regeneration of Nitrogenous Organic Matter in Sea Water. *Biol. Bull.*, Vol. LXXII, No. 2, pp. 165-175. 1937.

No. 130. H. C. Stetson and J. E. Upson. Bottom Deposits of the Ross Sea. *Jour. Sedimentary Petrology*, Vol. 7, No. 2, pp. 55–66. 1937.

No. 131. G. H. Parker. Integumentary Color Changes of Elasmobranch Fishes Especially of Mustelus. *Proc. Am. Philos. Soc.*, Vol. 77, No. 3, pp. 223–247. 1937.

No. 132. John L. Fuller. Feeding Rate of *Calanus finmarchicus* in Relation to Environmental Conditions. *Biol. Bull.*, Vol. LXXII, No. 2, pp. 233–246. 1937.

No. 133. Charles E. Renn. Bacteria and the Phosphorus Cycle in the Sea. *Biol. Bull.*, Vol. LXXII, No. 2, pp. 190–195. 1937.

No. 134. Robert C. Stauffer. Changes in the Invertebrate Community of a Lagoon after Disappearance of the Eel Grass. *Ecology*, Vol. XVIII, No. 3, pp. 427–431. 1937.

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No. 136. Marshall Schalk. A Textural Study of the Outer Beach of Cape Cod, Massachusetts. *Jour. Sedimentary Petrology*, Vol. 8, No. 2, pp. 41-54. 1938.

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No. 153. R. B. Montgomery. A Suggested Method for Representing Gradient Flow in Isentropic Surfaces. *Bull. Am. Meteorol. Soc.*, Vol. 18, pp. 210–212. 1937.

No. 154. Floyd M. Soule. Oceanography. Excerpt from International Ice Observation and Ice Patrol Service in the North Atlantic Ocean, Season of 1936. *U. S. Coast Guard Bull.*, No. 26, pp. 33-82. 1938.

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